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(54) Title: MODIFIED ANTIBODIES TO PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND USES THEREOF

(57) Abstract: Modified antibodies, or antigen-binding fragments thereof, to the extracellular domain of human prostate specific membrane antigen (PSMA) are provided. The modified anti-PSMA antibodies, or antigen-binding fragments thereof, have been rendered less immunogenic compared to their unmodified counterparts to a given species, e.g., a human. Pharmaceutical compositions including the aforesaid antibodies, nucleic acids, recombinant expression vectors and host cells for making such antibodies and fragments are also disclosed. Methods of using the antibodies of the invention to detect human PSMA, or to ablate or kill a PSMA-expressing cell, e.g., a PSMA-expressing cancer or prostatic cell, either *in vitro* or *in vivo*, are also provided.

MODIFIED ANTIBODIES TO PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND USES THEREOF

RELATED APPLICATIONS

This application claims priority to U.S. provisional application number 60/295,214 filed on June 1, 2001, 60/323,585 filed on September 20, 2001, and 60/362,810 filed on March 8, 2002, the contents of all of which are incorporated herein by reference. This invention was made with government support under Department of Defense Grant number DAMD17-98-1-8594. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0001] The present invention relates to antibodies, e.g., modified, e.g., deimmunized, antibodies, to the extracellular domain of human prostate specific membrane antigen (PSMA) and their uses in treating, preventing, and diagnosing prostatic disorders and cancers.

BACKGROUND OF THE INVENTION

[0002] Prostate cancer is one of the most common causes of cancer deaths in American males. In 1999, approximately 185,000 new cases were diagnosed and 37,500 died of this disease (NCI SEER data). It accounts for about 40% of all cancers diagnosed in men. A male born in the U.S. in 1990 has approximately a 1 in 8 likelihood of being diagnosed with clinically apparent prostate cancer in his lifetime. Even prior to the recent increase in incidence, prostate cancer was the most prevalent cancer in men (Feldman, A.R. et al. (1986) *NEJM* 315:1394-7).

[0003] There is currently very limited treatment for prostate cancer once it has metastasized (spread beyond the prostate). Currently, systemic therapy is limited to various forms of androgen (male hormone) deprivation. While most patients will demonstrate initial clinical improvement, virtually inevitably, androgen-independent cells develop. Endocrine therapy is thus palliative, not curative. In a study of 1387 patients with metastatic disease detectable by imaging (e.g., bone or CT scan), the median time to objective disease progression (excluding biochemical/PSA progression) after initiation of hormonal therapy (i.e., development of androgen-independence) was 16-48 months (Eisenberger M.A., et al. (1998) *NEJM* 339:1036-42). Median overall survival in these patients was 28-52 months from the onset of hormonal treatment (Eisenberger M.A., et al. (1998) *supra.*). Subsequent to developing androgen-independence, there is no effective standard therapy and the median duration of survival is 9-12

months (Vollmer, R.T., et al. (1999) *Clin Can Res* 5: 831-7; Hudes G., et al., (1997) *Proc Am Soc Clin Oncol* 16:316a (abstract); Pienta K.J., et al. (1994) *J Clin Oncol* 12(10):2005-12; Pienta K.J., et al. (1997) *Urology* 50:401-7; Tannock I.F., et al., (1996) *J Clin Oncol* 14:1756-65; Kantoff P.W., et al., (1996) *J. Clin. Oncol.* 15 (Suppl):25:110-25). Cytotoxic chemotherapy is poorly tolerated in this age group and generally considered ineffective and/or impractical. In addition, prostate cancer is relatively resistant to cytotoxic agents. Thus, chemotherapeutic regimen has not demonstrated a significant survival benefit in this patient group.

[0004] For men with a life expectancy of less than 10 years, watchful waiting is appropriate where low-grade, low-stage prostate cancer is discovered at the time of a partial prostatectomy for benign hyperplasia (W.J. Catalona, (1994) *New Engl. J. Med.*, 331(15):996-1004). Such cancers rarely progress during the first five years after detection. On the other hand, for younger men, curative treatment is often more appropriate.

[0005] Where prostate cancer is localized and the patient's life expectancy is 10 years or more, radical prostatectomy offers the best chance for eradication of the disease. Historically, the drawback of this procedure is that most cancers had spread beyond the bounds of the operation by the time they were detected. However, the use of prostate-specific antigen testing has permitted early detection of prostate cancer. As a result, surgery is less extensive with fewer complications. Patients with bulky, high-grade tumors are less likely to be successfully treated by radical prostatectomy.

[0006] After surgery, if there are detectable serum prostate-specific antigen concentrations, persistent cancer is indicated. In many cases, prostate-specific antigen concentrations can be reduced by radiation treatment. However, this concentration often increases again within two years.

[0007] Radiation therapy has also been widely used as an alternative to radical prostatectomy. Patients generally treated by radiation therapy are those who are older and less healthy and those with higher-grade, more clinically advanced tumors. Particularly preferred procedures are external-beam therapy which involves three dimensional, conformal radiation therapy where the field of radiation is designed to conform to the volume of tissue treated; interstitial-radiation therapy where seeds of radioactive compounds are implanted using ultrasound guidance; and a combination of external-beam therapy and interstitial-radiation therapy.

[0008] For treatment of patients with locally advanced disease, hormonal therapy before or following radical prostatectomy or radiation therapy has been utilized. Hormonal therapy is the main form of treating men with disseminated prostate cancer. Orchiectomy reduces serum testosterone concentrations, while estrogen treatment is similarly beneficial. Diethylstilbestrol from estrogen is another useful hormonal therapy which has a disadvantage of causing cardiovascular toxicity. When either LHRH agonists, such as leuprolide, buserelin, or goserelin, or gonadotropin-releasing hormone antagonists, such as Abarelix, are administered testosterone concentrations are ultimately reduced. Flutamide and other nonsteroidal, anti-androgen agents block binding of testosterone to its intracellular receptors. As a result, it blocks the effect of testosterone, increasing serum testosterone concentrations and allows patients to remain potent - a significant problem after radical prostatectomy and radiation treatments.

[0009] In view of the shortcoming of existing therapies, there exists a need for improved modalities for preventing and treating cancers, such as prostate cancer.

SUMMARY OF THE INVENTION

[0010] This invention provides, *inter alia*, antibodies and particularly, modified antibodies, or antigen-binding fragments thereof, that bind to the extracellular domain of human prostate specific membrane antigen (PSMA). The modified anti-PSMA antibodies, or antigen-binding fragments thereof, have been rendered less immunogenic compared to their unmodified counterparts to a given species, e.g., a human. The modified anti-PSMA antibodies, or fragments thereof, bind to human PSMA with high affinity and specificity, and thus can be used as diagnostic, prophylactic, or therapeutic agents *in vivo* and *in vitro*. Accordingly, the invention provides antibodies and particularly modified anti-PSMA antibodies, antibody fragments, and pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such antibodies and fragments. Methods of using the antibodies of the invention to detect PSMA, or to ablate or kill a PSMA-expressing cell, e.g., a PSMA-expressing cancer, a prostatic, or a vascular cell, either *in vitro* or *in vivo*, are also encompassed by the invention. Preferably, the modified antibodies are those having one or more complementarity determining regions (CDRs) from a J591, J415, J533 or E99 antibody. As discussed herein, the modified antibodies can be CDR-grafted, humanized, deimmunized, or, more generally, antibodies having the CDRs from a non-human antibody, e.g., murine J591, J415, J533 or E99

antibody, and a framework that is selected as less immunogenic in humans, e.g., less antigenic than the murine frameworks in which a murine CDR naturally occurs.

[0011] The antibodies, e.g., modified antibodies of the invention interact with, e.g., bind to, PSMA, preferably human PSMA, with high affinity and specificity. For example, the antibody binds to human PSMA with an affinity constant of at least 10^7 M^{-1}, preferably between 10^8 M^{-1} and 10^{10} M^{-1}, or about 10^9 M^{-1}. Preferably, the antibody interacts with, e.g., binds to, the extracellular domain of PSMA, and most preferably, the extracellular domain of human PSMA (e.g., amino acids 44-750 of human PSMA).

[0012] In some embodiments, the anti-PSMA antibody binds all or part of an epitope bound by an antibody described herein, e.g., a J591, E99, J415, and J533 antibody. The anti-PSMA antibody can inhibit, e.g., competitively inhibit, the binding of an antibody described herein, e.g., a J591, E99, J415, and J533 antibody, to human PSMA. An anti-PSMA antibody may bind to an epitope, e.g., a conformational or a linear epitope, which epitope when bound prevents binding of an antibody described herein, e.g., a J591, E99, J415, and J533 antibody. The epitope can be in close proximity spatially or functionally-associated, e.g., an overlapping or adjacent epitope in linear sequence or conformational space, to the one recognized by the J591, E99, J415, or J533 antibody.

[0013] In some embodiments, the anti-PSMA antibody binds to an epitope located wholly or partially within the region of about amino acids 120 to 500, preferably 130 to 450, more preferably, 134 to 437, or 153 to 347, of human PSMA. Preferably, the epitope includes at least one glycosylation site, e.g., at least one N-linked glycosylation site (e.g., the asparagine residue located at about amino acids 190-200, preferably at about amino acid 195, of human PSMA).

[0014] Human PSMA is expressed on the surface of normal, benign hyperplastic, and cancerous prostate epithelial cells, as well as vascular endothelial cells proximate to cancerous cells, e.g., renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), pancreatic (e.g., pancreatic duct), melanoma (e.g., malignant melanoma), or soft tissue sarcoma cancerous cells. The expression of human PSMA is substantially lower on non-malignant prostate cells where PSM', a splice variant that lacks a portion of the N-terminal domain that includes the transmembrane domain, is more abundant. Due to the absence of the N-terminal region

containing the transmembrane domain, PSM' is primarily cytoplasmic and is not located on the cell membrane. The antibodies, e.g., the modified antibodies, of the invention bind to the cell surface of cells that express PSMA. PSMA is normally recycled from the cell membrane into the cell. Thus, the antibodies of the invention are internalized with PSMA through the process of PSMA recirculation, thereby permitting delivery of an agent conjugated to the antibody, e.g., a labeling agent, a cytotoxic agent, or a viral particle (e.g., a viral particle containing genes that encode cytotoxic agents, e.g., apoptosis-promoting factors). Accordingly, antibodies, e.g., modified antibodies, described herein, can be used to target living normal, benign hyperplastic, and cancerous prostate epithelial cells, as well as vascular endothelial cells proximate to cancerous cells.

[0015] An antibody, e.g., a modified antibody, is preferably monospecific, e.g., a monoclonal antibody, or an antigen-binding fragment thereof. The antibodies, e.g., the modified antibodies, can be full-length (e.g., an IgG (e.g., an IgG1, IgG2, IgG3, IgG4), IgM, IgA (e.g., IgA1, IgA2), IgD, and IgE, but preferably an IgG) or can include only an antigen-binding fragment (*e.g.*, a Fab, $F(ab')_2$ or scFv fragment, or one or more CDRs). An antibody, or antigen-binding fragment thereof, can include two heavy chain immunoglobulins and two light chain immunoglobulins, or can be a single chain antibody. The antibodies can, optionally, include a constant region chosen from a kappa, lambda, alpha, gamma, delta, epsilon or a mu constant region gene. A preferred anti-PSMA antibody includes a heavy and light chain constant region substantially from a human antibody, e.g., a human IgG1 constant region, a portion thereof, or a consensus sequence.

[0016] In a preferred embodiment, the antibodies (or fragments thereof) are recombinant or modified anti-PSMA antibodies chosen from, e.g., a chimeric, a humanized, a deimmunized, or an *in vitro* generated antibody. In other embodiments, the anti-PSMA antibodies are human antibodies. In one embodiment, a modified antibody of the invention is a deimmunized anti-PSMA antibody, e.g., a deimmunized form of E99, J415, J533 or J591 (e.g., a deimmunized form of an antibody produced by a hybridoma cell line having an ATCC Accession Number HB-12101, HB-12109, HB-12127, and HB-12126, respectively). Preferably, a modified antibody is a deimmunized form of J591 or J415 (referred to herein as "deJ591" or "deJ415", respectively). Most preferably, the antibody is a deimmunized form of J591.

[0017] Any combination of anti-PSMA antibodies is within the scope of the invention, e.g., two or more antibodies that bind to different regions of PSMA, e.g., antibodies that bind to two different epitopes on the extracellular domain of PSMA.

[0018] In some embodiments, the anti-PSMA antibody, e.g., the modified anti-PSMA antibody or antigen-binding fragment thereof, includes at least one light or heavy chain immuglobulin (or preferably, at least one light chain immuglobulin and at least one heavy chain immuglobulin). Preferably, each immunoglobulin includes a light or a heavy chain variable region having at least one, two and, preferably, three CDRs substantially identical to a CDR from a non-human anti-PSMA light or heavy chain variable region, respectively. For example, the antibody or antigen-binding fragment thereof can have at least one, two and preferably three CDRs from: the heavy chain variable region of murine J591 (see SEQ ID NO:1, 2, and 3, depicted in Figure 1A); the light chain variable region of murine J591 (see SEQ ID NO:4, 5, and 6, depicted in Figure 1B); the heavy chain variable region of murine J415 (see SEQ ID NO:29, 30, and 31, depicted in Figure 5); the light chain variable region of murine J415 (see SEQ ID NO:32, 33, and 34, depicted in Figure 6); the heavy chain variable region of murine J533 (see SEQ ID NO:93, 94, and 95, depicted in Figure 9A); the light chain variable region of murine J533 (see SEQ ID NO:96, 97, and 98, depicted in Figure 10A); the heavy chain variable region of murine E99 (see SEQ ID NO:99, 100, and 101, depicted in Figure 11A); or the light chain variable region of murine E99 (see SEQ ID NO:102, 103, and 104, depicted in Figure 12A). In other embodiments, the modified antibody or antigen-binding fragment thereof can have at least one, two, and preferably three CDRs from the light or heavy chain variable region of the antibody produced by the cell line having ATCC Accession Number HB-12126 or the deimmunized J591 antibody produced by the cell line having ATCC Accession Number PTA-3709. In other embodiments, the modified antibody or antigen-binding fragment thereof can have at least one, two and preferably three CDRs from the light or heavy chain variable region of the antibody produced by the cell line having ATCC Accession Number HB-12109 or the deimmunized J415 antibody produced by a cell line having ATCC Accession Number PTA-4174. In still other embodiments, the modified antibody or antigen-binding fragment thereof can have at least one, two and preferably three CDRs from the light or heavy chain variable region of the antibody produced by the cell line having ATCC Accession Number HB-12127 or the antibody produced by a cell line having ATCC Accession Number HB-12101.

[0019] In one preferred embodiment, the modified antibody or antigen-binding fragment thereof includes all six CDRs from the same non-human anti-PSMA antibody, e.g., a murine J591, J415, J533 or E99 antibody. In some embodiments, the CDRs have the amino acid sequences of SEQ ID NO:1, 2, 3, 4, 5 and 6 (corresponding to murine J591 heavy and light chain CDRs), the amino acid sequences of the CDRs of the antibody produced by the cell line having ATCC Accesion number HB-12126, or the deimmunized J591 antibody produced by the cell line having ATCC Accession Number PTA-3709, or sequences substantially identical thereto. In other embodiments, the CDRs have the amino acid sequences of SEQ ID NO:29, 30, 31, 32, 33, and 34 (corresponding to murine J415 heavy and light chain CDRs), the amino acid sequences of the CDRs of the antibody produced by the cell line having ATCC Accession Number HB-12109, or the deimmunized J415 antibody produced by the cell line having ATCC Accession Number PTA-4174, or sequences substantially identical thereto. In other embodiments, the CDRs have the amino acid sequences of SEQ ID NO:93, 94, 95, 96, 97, and 98 (corresponding to murine J533 heavy and light chain CDRs), the amino acid sequences of the CDRs of the antibody produced by the cell line having ATCC Accession Number HB-12127, or sequences substantially identical thereto. In still other embodiments, the CDRs have the amino acid sequences of SEQ ID NO:99, 100, 101, 102, 103, and 104 (corresponding to murine E99 heavy and light chain CDRs), the amino acid sequences of the CDRs of the antibody produced by the cell line having ATCC Accession Number HB-12101, or sequences substantially identical thereto.

[0020] The amino acid sequence of the CDRs for antibodies J591, J415, J533 and E99 are provided below in Table 1.

Table 1: CDR Sequences

NAME	Organism	FIG.	SEQ ID NO:	SEQUENCE
V_H CDR1 J591	Mus musculus	Fig. 1A	1	GYTFTEYTIH
V_H CDR2 J591	Mus musculus	Fig. 1A	2	NINPNNGGTTYNQKFED
V_H CDR3 J591	Mus musculus	Fig. 1A	3	GWNFDY
V_L CDR1	Mus musculus	Fig. 1B	4	KASQDVGTAVD

J591				
V_L CDR2 J591	Mus musculus	Fig. 1B	5	WASTRHT
V_L CDR3 J591	Mus musculus	Fig. 1B	6	QQYNSYPLT
V_H CDR1 J415	Mus musculus	Fig. 5	29	GFTFSNYWMN
V_H CDR2 J415	Mus musculus	Fig. 5	30	EIRSQSNNFATHYAESVKG
V_H CDR3 J415	Mus musculus	Fig. 5	31	RWNNF
V_L CDR1 J415	Mus musculus	Fig. 6	32	KASENVGTYVS
V_L CDR2 J415	Mus musculus	Fig. 6	33	GASNRFT
V_L CDR3 J415	Mus musculus	Fig. 6	34	GQSYTFPYT
V_H CDR1 J533	Mus musculus	Fig. 9A	93	GYTFTGYVMH
V_H CDR2 J533	Mus musculus	Fig. 9A	94	YINPYNDVTRYNGKFKG
V_H CDR3 J533	Mus musculus	Fig. 9A	95	GENWYYFDS
V_L CDR1 J533	Mus musculus	Fig. 10A	96	RASESIDSYDNTFMH
V_L CDR2 J533	Mus musculus	Fig. 10A	97	RASILES
V_L CDR3 J533	Mus musculus	Fig. 10A	98	HQSIEDPYT
V_H CDR1 E99	Mus musculus	Fig. 11A	99	GFSLTAYGIN
V_H CDR2 E99	Mus musculus	Fig. 11A	100	VIWPDGNTDYNSTLKS
V_H CDR3 E99	Mus musculus	Fig. 11A	101	DSYGNFKRGWFDF
V_L CDR1 E99	Mus musculus	Fig. 12A	102	KASQNVGSDVA
V_L CDR2 E99	Mus musculus	Fig. 12A	103	STSYRYS
V_L CDR3 E99	Mus musculus	Fig. 12A	104	QQYNSYPLT

[0021] The light or heavy chain immunoglobulin of the modified anti-PSMA antibody or antigen-binding fragment thereof can further include a light chain or a heavy chain variable framework sequence from a light chain or heavy chain variable framework present in a human or a non-human, e.g., rodent, antibody (e.g., the murine J591, J415, J533 or E99 antibody heavy

chain or light chain variable framework). In some embodiments, the light chain or the heavy chain variable framework can be chosen from:

i a light or heavy chain variable framework including at least 5, 10, 20, 30, 40, 50, 60, 70, or 80 amino acid residues from a human light or heavy chain variable framework, e.g., a light or heavy chain variable framework residue from a mature human antibody, a human germline antibody sequence, or a human consensus antibody sequence;

ii a light or heavy chain variable framework including at least 5, but less than 30, amino acid residues from a human light chain or heavy chain variable framework, e.g., a light chain or heavy chain variable framework residue from a mature human antibody, a human germline antibody sequence, or a human consensus antibody sequence;

iii a light or heavy chain variable framework including at least 5, 10, 20, 30, 40, 50, 60, 75 or more amino acid residues from a light or heavy variable framework from a non-human antibody, e.g., a murine antibody (e.g., an anti-PSMA antibody having the framework amino acid sequence shown in SEQ ID NO:7 or 8 (from the heavy and light chain, respectively, of murine J591; see Figures 1A and 1B), SEQ ID NO:35 or 36 (from the heavy and light chain, respectively, of murine J415; see Figures 5 and 6), SEQ ID NO:109 or 114 (from the heavy and light chain, respectively, of murine J533; see Figures 9A and 10A), or SEQ ID NO:119 or 124 (from the heavy and light chain, respectively, of murine E99; see Figures 11A and 12A), or the framework of a murine antibody described herein (e.g., a murine J591, J415, J533, or E99 antibody produced by a hybridoma cell line having an ATCC Accession Number HB-12126, HB-12109, HB-12127 or HB-12101);

iv a light or heavy chain variable framework, which has at least 60%, 65%, 70%, 72%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identity with, or which has an amino acid sequence which differs by at least 1, 2, 5, or more residues, but less than 10, 20, 30 or 40 residues from, the sequence of the framework of a light or heavy chain variable region of a non-human antibody, e.g., a murine antibody (e.g., an anti-PSMA antibody having the framework amino acid sequence shown in SEQ ID NO:7 or 8 (from the heavy and light chain, respectively, of murine J591; see Figures 1A and 1B), SEQ ID NO:35 or 36 (from the heavy and light chain, respectively, of murine J415; see Figures 5 and 6), SEQ ID NO:109 or 114 (from the heavy and light chain, respectively,

cell line having ATCC Accession Number PTA-3709 or PTA-4174. Preferably, the heavy or light chain framework region includes the amino acid sequence shown in SEQ ID NO:17 or SEQ ID NO:18, respectively, SEQ ID NO:45 or SEQ ID NO:46, respectively, or the heavy or light chain framework sequence of the antibody produced by the cell line having ATCC Accession Number PTA-3709 or PTA-4174.

[0023] In other embodiments, the heavy or light chain variable region of the modified anti-PSMA antibody has an amino acid sequence which has at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or more identity with SEQ ID NO:21 or SEQ ID NO:22, respectively (corresponding to the heavy and light chain variable regions of deimmunized J591; see Figures 2A-2B), SEQ ID NO:49 or SEQ ID NO:50, respectively (corresponding to the heavy and light chain variable regions of deimmunized J415, J415DIVH4 and J415DIVK5; see Figures 5 or 6), or the heavy or light chain variable region sequence of the antibody produced by the cell line having ATCC Accession Number PTA-3709 or PTA-4174. In other embodiments, the heavy or light chain variable region of the modified anti-PSMA antibody has an amino acid sequence that differs by at least 1, 2, 5, or more residues, but less than 10, 20, 30, or 40 residues, from the amino acid sequence of SEQ ID NO:21 or SEQ ID NO:22, respectively, SEQ ID NO:49 or SEQ ID NO:50, respectively, or the heavy or light chain variable region sequence of the antibody produced by the cell line having ATCC Accession Number PTA-3709 or PTA-4174. Preferably, the light or heavy chain variable region includes the amino acid sequence shown in SEQ ID NO:21 or SEQ ID NO:22, respectively, SEQ ID NO:49 or SEQ ID NO:50, respectively, or the heavy or light chain variable region sequence of the antibody produced by the cell line having ATCC Accession Number PTA-3709 or PTA-4174.

[0024] Preferred modified anti-PSMA antibodies include at least one, preferably two, light chain variable regions and at least one, preferably two, heavy chain variable regions having the amino acid sequence shown in SEQ ID NO:21 and SEQ ID NO:22, respectively (corresponding to the heavy and light chain variable regions of deimmunized J591; see Figures 2A-2B), SEQ ID NO:49 and SEQ ID NO:50, respectively (corresponding to the heavy and light chain variable regions of deimmunized J415, J415DIVH4 and J415DIVK5; see Figures 5 and 6), or at least one, preferably two, modified light chain variable region sequences and at least one, preferably two, heavy chain variable region sequences of the antibody produced by the cell line having ATCC Accession Number PTA-3709 or PTA-4174.

[0025] In other embodiments, the light or heavy chain variable framework of the anti-PSMA antibody, or antigen-binding fragment thereof, includes at least one, two, three, four, five, six, seven, eight, nine, ten, fifteen, sixteen, or seventeen amino acid residues from a human light or heavy chain variable framework, e.g., a light or heavy chain variable framework residue from a mature human antibody, a human germline antibody sequence, or a consensus antibody sequence.

[0026] In some embodiments, the amino acid residue from the human light chain variable framework is the same as the residue found at the same position in a human germline antibody seqence. Preferably, the amino acid residue from the human light chain variable framework is *the most common residue at the same position in the human germline antibody sequence.* Preferably, the light chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, has at least one, two, three, five, seven, ten amino acid residues which differ from the framework of the non-human anti-PSMA light chain variable region (e.g., the murine J591 light chain variable region), or which is from a human light chain variable framework (e.g., a human germline, mature, or consensus framework sequence), at a position selected from the group consisting of: residue 8, 9, 10, 11, 20, 22, 60, 63, 76, 77, 78, 80, 83, 87, 103, 104 and 106 (Kabat numbering as shown in Table 2). Preferably, the light chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, has at least one, two, three, five, seven, or ten amino acid residues from the human light chain variable framework selected from the group consisting of: residue 8 (proline), 9 (serine), 10 (serine), 11 (leucine), 20 (threonine), 22 (threonine), 60 (serine), 63 (serine), 76 (serine), 77 (serine), 78 (leucine), 80 (proline), 83 (phenylalanine), 87 (tyrosine), 103 (lysine), 104 (valine) and 106 (isoleucine) (Kabat numbering as shown in Table 2).

[0027] The amino acid replacements in the deimmunized J591 light chain variable region are provided below in Table 2. The left panel indicates the amino acid number according to Kabat, E.A., *et al.* (1991) supra; the middle panel indicates the replacements of the residue in the mouse sequence and the corresponding mouse residues; and the right panel indicates the most common residue in the corresponding postion in the human germline.

Table 2

Position Kabat No	*Substitution* of mouse sequence	*Most common in* human germline

3	V→Q	V
8	H→P	P
9	K→S	S
10	F→S	S
11	M→L	L
20	S→T	T
21	I→L	I
22	I→T	T
42	Q→P	K
58	V→I	V
60	D→S	S
63	T→S	S
76	T→S	S
77	T→S	S
78	V→L	L
80	S→P	P
83	L→F	F
87	F→Y	Y
100	A→P	Q
103	M→K	K
104	L→V	V
106	L→I	I

[0028] In other embodiments, the light chain variable framework of the anti-PSMA antibody, or antigen-binding fragment thereof, has at least one, two, three, five, or seven amino acid residues which differ from the framework of a non-human anti-PSMA light chain variable region (e.g., the murine J415 light chain variable region), or which is from a human light chain variable framework (e.g., a human germline, mature, or consensus framework), at a position selected from the group consisting of: residue 13, 15, 19, 41, 63, 68, and 80 (linear numbering as shown in Figure 6). Preferably, the light chain variable framework of the modified antibody, or antigen-binding fragment thereof, has at least one, two, three, five, or seven amino acid residues from the human consensus light chain variable framework selected from the group consisting of: residue 13 (alanine), 15 (alanine), 19 (methionine), 41 (threonine), 63 (serine), 68 (glycine), and 80 (alanine) (linear numbering as shown in Figure 6).

[0029] The amino acid replacements in the deimmunized J415 light chain variable region are provided below in Table 3. The left panel indicates the amino acid number using linear numbering; the middle panel indicates the replacements of the residue in the mouse sequence and

the corresponding mouse residues; and the right panel indicates the most common residue in the corresponding postion in the human germline.

Table 3

Position Linear No	Substitution of mouse sequence	Most common in human germline
13	I→A	A
15	V→A	A
19	V→M	M
41	E→T	T
63	T→S	S
68	A→G	G
80	T→A	A

[0030] In other embodiments, the light chain variable framework of the anti-PSMA antibody, or antigen-binding fragment thereof, includes at least 5, but no more than 80, amino acid residues from the light chain variable framework shown in SEQ ID NO:8 (from murine J591; see Figure 1B), SEQ ID NO:36 (from murine J415; see Figure 6), SEQ ID NO:114 (from murine J533; see Figure 10A), or SEQ ID NO:124 (from murine E99; see Figure 12A), or the light chain variable framework of an antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126, HB-12109, HB-12127 or HB-12101. Preferably, the light chain variable framework has at least 60%, 65%, 70%, 72%, 75%, 80%, 85%, 90%, or 94% identity with, or differs by at least 5, 7, 10, 20, or 30 but less than 10, 20, 30, or 40 amino acid residues from, the non-human light chain variable framework, e.g., the murine J591 or J415 light chain variable framework shown in SEQ ID NO:8 or SEQ ID NO:36, respectively, or the light chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126 or HB-12109. In other embodiments, the light chain variable framework is from murine J591 antibody (SEQ ID NO:8; see Figure 1B), from murine J415 antibody (SEQ ID NO:36; see Figure 6), from murine J533 antibody (SEQ ID NO:114; see Figure 10A), or from murine E99 antibody (SEQ ID NO:124; see Figure 12A), or the light chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126, HB-12109, HB-12127 or HB-12101.

[0031] In yet other embodiments, the light chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human (e.g., a murine)

light chain variable framework (e.g., a murine J591 light chain variable framework as shown in SEQ ID NO:8 or the light chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126) which has at least 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, or 23 amino acid replacements. In one embodiment, the non-human light chain variable framework includes one or more of:

a framework region 1 having at least 5, 6, 7, or 8 replacements;

a framework region 2 having at least one replacement;

a framework region 3 having at least 5, 6, 7, 8, or 9 replacements; or

a framework region 4 having at least 2, 3 or 4 replacements.

[0032] In yet other embodiments, the light chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human (e.g., a murine) light chain variable framework (e.g., a murine J415 light chain variable framework as shown in SEQ ID NO:36 or the light chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109) which has at least 1, 2, 3, 4, 5, 6, 7, 8, or 10 amino acid replacements. In some embodiments, the non-human light chain variable framework includes one or more of:

a framework region 1 having at least 1, 2 or 3 replacements;

a framework region 2 having at least one replacement; or

a framework region 3 having at least 1, 2 or 3 replacements.

[0033] The replacement can be selected from: a conservative substitution of a non-human residue, or a residue found in a human germline, mature or consensus framework sequence at the same position, e.g. the most common residue in the human germline sequence at the same position. In some embodiments, the light chain variable framework has at least 3, 4 and preferably 5 conservative substitutions. In other embodiments, the light chain variable framework has at least 5, 7, 10, 15, 16, or 17 amino acid replacements wherein the replacement amino acid residue is the most common residue in the human germline framework sequence at the same position.

[0034] In some embodiments, the non-human light chain variable framework has at least one, two, three, five, seven, ten, eleven, fifteen, sixteen, seventeen, nineteen, twenty, twenty-one or twenty-two amino acid replacements at a position selected from the group consisting of: residue 3, 8, 9, 10, 11, 20, 21, 22, 42, 58, 60, 63, 76, 77, 78, 80, 83, 87, 100, 103, 104 and 106

(Kabat numbering as shown in Table 2). The replacement can be chosen from one or more of: residue 3 (glutamine), 8 (proline), 9 (serine), 10 (serine), 11 (leucine), 20 (threonine), 21 (leucine), 22 (threonine), 42 (proline), 58 (isoleucine), 60 (serine), 63 (serine), 76 (serine), 77 (serine), 78 (leucine), 80 (proline), 83 (phenylalanine), 87 (tyrosine), 100 (proline), 103 (lysine), 104 (valine) and 106 (isoleucine) (Kabat numbering as shown in Table 2).

[0035] In other embodiments, the non-human light chain variable framework has at least one, two, three, five, or seven amino acid replacements at a position selected from the group consisting of: residue 13, 15, 19, 41, 63, 68 and 80 (linear numbering as shown in Table 3). Preferably, the light chain variable framework of the modified antibody, or antigen-binding fragment thereof, has at least one, two, three, five, seven amino acid residues from the human consensus light chain variable framework selected from the group consisting of: residue 13 (alanine), 15 (alanine), 19 (methionine), 41 (threonine), 63 (serine), 68 (glycine) and 80 (alanine) (linear numbering as shown in Table 3).

[0036] Preferably, the heavy chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, has at least one, two, three, five, seven, or eight amino acid residues, which differ from the framework of the non-human anti-PSMA heavy chain variable region (e.g., the murine J591 heavy chain variable region), or which is from a human heavy chain variable framework (e.g., a human germline framework), at a position selected from the group consisting of: residue 5, 40, 41, 44, 82a, 83, 87, and 108 (Kabat numbering as shown in Table 4). Preferably, the heavy chain variable framework of the recombinant antibody, or antigen-binding fragment thereof, has at least one amino acid residue from the human heavy chain variable framework selected from the group consisting of: residue 5 (valine), 40 (alanine), 41 (proline), 44 (glycine), 82a (serine), 83 (arginine), 87 (threonine), or 108 (leucine) (Kabat numbering as shown in Table 4).

[0037] The amino acid replacements in the deimmunized J591 heavy chain variable region are provided below in Table 4. The left panel indicates the amino acid number according to Kabat, E.A., et al. (1991) supra; the middle panel indicates the replacements of the residue in the mouse sequence and the corresponding mouse residues; and the right panel indicates the most common residue in the corresponding postion in the human germline.

Table 4

Position Kabat No.	Substitution of mouse sequence	Most common in human germline
5	Q→V	V
11	L→V	L
12	V→K	V
16	T→A	G
17	S→T	S
19	R→K	R
40	S→A	A
41	H→P	P
44	S→G	G
75	S→T	K
76	S→D	N
82a	R→S	S
83	T→R	R
87	S→T	T
108	T→L	L

[0038] In other embodiments, the heavy chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, has at least one, two, three, four, five amino acid residues, which differ from the framework of a non-human anti-PSMA heavy chain variable region (e.g., the murine J415 heavy chain variable region), or which is from a human heavy chain variable framework (e.g., a human mature, consensus, or germline framework), at a position selected from the group consisting of: residue 20, 87, 94, 95, and 112 (linear numbering as shown in Table 5). Preferably, the heavy chain variable framework of the recombinant antibody, or antigen-binding fragment thereof, has at least one, two, three, four, five amino acid residues from the human heavy chain variable framework selected from the group consisting of: residue 20 (isoleucine), 87 (serine), 94 (alanine), 95 (valine), and 112 (valine) (linear numbering as shown in Table 5).

[0039] The amino acid replacements in the deimmunized J415 heavy chain variable region are provided below in Table 5. The left panel indicates the linear amino acid number; the middle panel indicates the replacements of the residue in the mouse sequence and the corresponding mouse residues; and the right panel indicates the most common residue in the corresponding postion in the human germline.

Table 5

Position	Substitution	Most common in

Kabat No	of mouse sequence	human germline
20	L→I	I
87	N→S	S
94	G→A	A
95	I→V	V
112	L→V	V

[0040] In other embodiments, the heavy chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes at least 5 but no more than 75 or 82 amino acid residues from the heavy chain variable framework shown in SEQ ID NO:7 (from murine J591; see Figure 1A), SEQ ID NO:35 (from murine J415; see Figure 5), SEQ ID NO:109 (from murine J533; see Figure 9A), or SEQ ID NO:119 (from murine E99; see Figure 11A), or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126, HB-12109, HB-12127 or HB-12101. Preferably, the heavy chain variable framework has at least 60%, 65%, 70%, 80%, 82%, 85%, 90%, or 94% identity with, or differs by at least 5, 10, 20, or 30 but less than 10, 20, 30, or 40 residues from, a non-human heavy chain variable framework, e.g., the murine J591 or J415 or heavy chain variable framework shown in SEQ ID NO:7 or SEQ ID NO:35, respectively, or a heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126 or 12109. In other embodiments, the non-human heavy chain variable framework is from murine J591 antibody (SEQ ID NO:7; see Figure 1A), from murine J415 antibody (SEQ ID NO:35; see Figure 5), from murine J533 antibody (SEQ ID NO:109; see Figure 9A), or from murine E99 antibody (SEQ ID NO:119; see Figure 11A), or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126, HB-12109, HB-12127 or HB-12101.

[0041] In yet other embodiments, the heavy chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human (e.g., a murine) heavy chain variable framework (e.g., a murine J591 heavy chain variable framework (SEQ ID NO:7, as shown Figure 1A, or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126) which has at least 3, 5, 10, 15, 16, 17, 18, or 19 amino acid replacements. In one embodiment, the non-human heavy chain variable framework of the modified anti-PSMA antibody includes one or more of:

a framework region 1 having at least 4, 5, or 6 replacements;

a framework region 2 having at least 1, 2, or 3 replacements;

a framework region 3 having at least 3, 4, or 5 replacements; or

a framework region 4 having at least one replacement.

[0042] In yet other embodiments, the heavy chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human (e.g., a murine) heavy chain variable framework (e.g., a murine J415 heavy chain variable framework (SEQ ID NO:35, as shown in Figure 5, or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109) which has at least 1, 2, 3, 4, or 5 amino acid replacements. In one embodiment, the non-human heavy chain variable framework of the modified anti-PSMA antibody includes one or more of:

a framework region 1 having at least one replacement;

a framework region 3 having at least 1, 2, or 3 replacements; or

a framework region 4 having at least one replacement.

[0043] The replacement can be chosen from: a conservative substitution of a non-human residue, or a residue found in a human germline, mature or consensus sequence at the same position, e.g. the most common residue in the human germline at the same position. In one embodiment, the heavy chain variable framework has at least 3, 4, 5, 6 and preferably 7 conservative substitutions. Preferably, the heavy chain variable framework has at least 5, 6, 7 and preferably 8 replacements by the most common residue in the human germline at the same position.

[0044] In some embodiments, the non-human heavy chain variable framework has at least one amino acid replacement at a position selected from the group consisting of: residue 5, 11, 12, 16, 17, 19, 40, 41, 44, 75, 76, 82a, 83, 87, and 108 (Kabat numbering as shown in Table 3). The replacement can be chosen from one or more of: 5 (valine), 11 (valine), 12 (lysine), 16 (alanine), 17 (threonine), 19 (lysine), 40 (alanine), 41 (proline), 44 (glycine), 75 (threonine), 76 (aspartate), 82a (serine), 83 (arginine), 87 (threonine), and 108 (leucine) (Kabat numbering as shown in Table 4).

[0045] In other embodiments, the non-human heavy chain variable framework has at least one amino acid replacement at a position selected from the group consisting of: residue 20, 87, 94, 95 and 112 (linear numbering as shown in Table 5). The replacement can be chosen from

one or more of: residue 20 (isoleucine), 87 (serine), 94 (alanine), 95 (valine), and 112 (valine) (linear numbering as shown in Table 5).

[0046] The amino acid sequence of the framework regions of the light and heavy chains regions of antibodies J591, J415, J533 and E99 are provided in Table 6, below.

Table 6: Framework Sequences

NAME	Organism	FIG.	SEQ ID NO:	SEQUENCE
V_H FR1-FR4 J591	Mus musculus	Fig. 1A	7	EVQLQQSGPELKKPGTSVRISCK TSWVKQSHGKSLEWIGKATLTV DKSSSTAYMELRSLTSEDSAVY YCAAWGQGTTLTVSS
V_L FR1-FR4 J591	Mus musculus	Fig. 1B	8	DIVMTQSHKFMSTSVGDRVSIIC WYQQKPGQSPKLLIYGVPDRFT GSGSGTDFTLTITNVQSEDLADY FCFGAGTMLDLK
V_H FR1 (Deimm) J591	Artificial - deimmunized heavy chain J591	Fig. 2A	9	EVQLVQSGPEVKKPGATVKISC KTS
V_H FR2 (Deimm) J591	Artificial - deimmunized heavy chain J591	Fig. 2A	10	WVKQAPGKGLEWIG
V_H FR3 (Deimm) J591	Artificial - deimmunized heavy chain J591	Fig. 2A	11	KATLTVDKSTDTAYMELSSLRS EDTAVYYCAA
V_H FR4 (Deimm) J591	Artificial - deimmunized heavy chain J591	Fig. 2A	12	WGQGTLLTVSS
V_L FR1 (Deimm) J591	Artificial - deimmunized light chain J591	Fig. 2B	13	DIQMTQSPSSLSTSVGDRVTLTC
V_L FR2 (Deimm) J591	Artificial - deimmunized light chain J591	Fig. 2B	14	WYQQKPGPSPKLLIY
V_L FR3 (Deimm) J591	Artificial - deimmunized light chain J591	Fig. 2B	15	GIPSRFSGSGSGTDFTLTISSLQPE DFADYYC

V_L FR4 (Deimm) **J591**	Artificial - deimmunized light chain J591	Fig. 2B	16	FGPGTKVDIK
V_H FR1-FR4 (Deimm) **J591**	Artificial - deimmunized heavy chain J591	Fig. 2A	17	EVQLVQSGPEVKKPGATVKISC KTSWVKQAPGKGLEWIGKATLT VDKSTDTAYMELSSLRSEDTAV YYCAAWGQGTLLTVSS
V_L FR1-FR4 (Deimm) **J591**	Artificial - deimmunized light chain J591	Fig. 2B	18	DIQMTQSPSSLSTSVGDRVTLTC WYQQKPGPSPKLLIYGIPSRFSGS GSGTDFTLTISSLQPEDFADYYC FGPGTKVDIK
V_H FR1-FR4 **J415**	Mus musculus	Fig. 5	35	EVKLEESGGGLVQPGGSMKLSC VASWVRQSPEKGLEWVARVIIS RDDSKSSVYLQMNNLRAEDTGI YYCTRWGQGTTLTVSS
V_L FR1-FR4 **J415**	Mus musculus	Fig. 6	36	NIVMTQFPKSMSISVGERVTLTC WYQQKPEQSPKMLIYGVPDRFT GSGSATDFILTISSVQTEDLVDY YCFGGGTKLEMK
V_H FR1 (Deimm) **J415-4**	Artificial - deimmunized heavy chain J415-4	Fig. 5	37	EVKLEESGGGLVQPGGSMKISC VAS
V_H FR2 (Deimm) **J415-4**	Artificial - deimmunized heavy chain J415-4	Fig. 5	38	WVRQSPEKGLEWVA
V_H FR3 (Deimm) **J415-4**	Artificial - deimmunized heavy chain J415-4	Fig. 5	39	RVIISRDDSKSSVYLQMNSLRAE DTAVYYCTR
V_H FR4 (Deimm) **J415-4**	Artificial - deimmunized heavy chain J415-4	Fig. 5	40	WGQGTTVTVSS
V_L FR1 (Deimm) **J415-5**	Artificial - deimmunized light chain J415-5	Fig. 6	41	NIVMTQFPKSMSASAGERMTLT C
V_L FR2 (Deimm) **J415-5**	Artificial - deimmunized light chain J415-5	Fig. 6	42	WYQQKPTQSPKMLIY
V_L FR3 (Deimm) **J415-5**	Artificial - deimmunized light chain J415-5	Fig. 6	43	GVPDRFSGSGSGTDFILTISSVQA EDLVDYYC

V_L FR4 (Deimm) **J415-5**	Artificial - deimmunized light chain J415-5	Fig. 6	44	FGGGTKLEMK
V_H FR1-FR4 (Deimm) **J415-4**	Artificial - deimmunized heavy chain J415-4	Fig. 5	45	EVKLEESGGGLVQPGGSMKISC VASWVRQSPEKGLEWVARVIIS RDDSKSSVYLQMNSLRAEDTAV YYCTRWGQGTTVTVSS
V_L FR1-FR4 (Deimm) **J415-5**	Artificial - deimmunized light chain J415-5	Fig. 6	46	NIVMTQFPKSMSASAGERMTLT CWYQQKPTQSPKMLIYGVPDRF SGSGSGTDFILTISSVQAEDLVDY YCFGGGTKLEMK
V_H FR1 **J533**	Mus musculus	Fig. 9A	105	EVQLQQSGPELVKPGASVKMSC KAS
V_H FR2 **J533**	Mus musculus	Fig. 9A	106	WVKQKPGQVLEWIG
V_H FR3 **J533**	Mus musculus	Fig. 9A	107	KATLTSDKYSSTAYMELSGLTSE DSAVYYCAR
V_H FR4 **J533**	Mus musculus	Fig. 9A	108	WGRGATLTVSS
V_H FR1-FR4 **J533**	Mus musculus	Fig. 9A	109	EVQLQQSGPELVKPGASVKMSC KASWVKQKPGQVLEWIGKATLT SDKYSSTAYMELSGLTSEDSAV YYCARWGRGATLTVSS
V_L FR1 **J533**	Mus musculus	Fig. 10A	110	DIVLTQSPASLAVSLGQRATISC
V_L FR2 **J533**	Mus musculus	Fig. 10A	111	WYQQKPGQPPNLLIF
V_L FR3 **J533**	Mus musculus	Fig. 10A	112	GIPARFSGSGSGTDFTLTIYPVEA DDVATYYC
V_L FR4 **J533**	Mus musculus	Fig. 10A	113	FGGGTKLEIK

V_L FR1-FR4 **J533**	Mus musculus	Fig. 10A	114	DIVLTQSPASLAVSLGQRATISC WYQQKPGQPPNLLIFGIPARFSG SGSGTDFTLTIYPVEADDVATYY CFGGGTKLEIK
V_H FR1 **E99**	Mus musculus	Fig. 11A	115	QVQLKESGPGLVASSQSLSITCT VS
V_H FR2 **E99**	Mus musculus	Fig. 11A	116	WVRQPPGKGLEWLG
V_H FR3 **E99**	Mus musculus	Fig. 11A	117	RLNIFKDNSKNQVFLKMSSFQTD DTARYFCAR
V_H FR4 **E99**	Mus musculus	Fig. 11A	118	WGQGTTLTVSS
V_H FR1-FR4 **E99**	Mus musculus	Fig. 11A	119	QVQLKESGPGLVASSQSLSITCT VSWVRQPPGKGLEWLGRLNIFK DNSKNQVFLKMSSFQTDDTARY FCARWGQGTTLTVSS
V_L FR1 **E99**	Mus musculus	Fig. 12A	120	NIVMTQSQKFMSTSPGDRVRVT C
V_L FR2 **E99**	Mus musculus	Fig. 12A	121	WYQAKPGQSPRILIY
V_L FR3 **E99**	Mus musculus	Fig. 12A	122	GVPDRFTAYGSGTDFTLTITNVQ SEDLTEYFC
V_L FR4 **E99**	Mus musculus	Fig. 12A	123	FGAGTKLELK
V_L FR1-FR4 **E99**	Mus musculus	Fig. 12A	124	NIVMTQSQKFMSTSPGDRVRVT CWYQAKPGQSPRILIYGVPDRFT AYGSGTDFTLTITNVQSEDLTEY FCFGAGTKLELK

[0047] In other embodiments, the anti-PSMA antibody, or antigen-binding fragment thereof, includes at least one light chain or heavy chain immuglobulin or, preferably, at least one light chain immuglobulin and at least one heavy chain immuglobulin. Preferably, the light chain

immunoglobulin includes a non-human light chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA light chain variable region (e.g., the murine J591 or J415 light chain variable region shown in SEQ ID NO:20 (see Figure 1B) or SEQ ID NO:48 (see Figure 6), respectively, or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126 or 12109) and a light chain framework which differs from the framework of the non-human, e.g., murine, anti-PSMA light chain framework (e.g., the murine J591 of J415 light chain framework shown in SEQ ID NO:8 (see Figure 1B) or SEQ ID NO:36 (see Figure 6), respectively, or the light chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126 or 12109) at one, two, three, four, five, six, seven or more positions selected from the group consisting of: residue 3, 8, 9, 10, 11, 20, 21, 22, 42, 58, 60, 63, 76, 77, 78, 80, 83, 87, 100, 103, 104 and 106 (Kabat numbering as in Table 2), or residues 13, 15, 19, 41, 63, 68, and 80 (linear numbering as in Table 3).

[0048] In other preferred embodiments, the heavy chain immunoglobulin includes a non-human heavy chain variable region comprising three complementarity determining regions (CDRs) from a non-human, e.g., murine, anti-PSMA heavy chain variable region (e.g., the murine J591 or J415 heavy chain variable region shown in SEQ ID NO:19 (see Figure 1A) or SEQ ID NO:47 (see Figure 5), respectively, or the heavy chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126 or HB-12109) and a modified heavy chain framework which differs from the framework of the non-human, e.g., murine, anti-PSMA heavy chain framework (e.g., the murine J591 or J415 heavy chain framework shown in SEQ ID NO:7 (see Figure 1A) or SEQ ID NO:35 (see Figure 5), respectively, or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126 or HB-12109) at one, two, three, four, five or more positions selected from the group consisting of: residue 5, 11, 12, 16, 17, 19, 40, 41, 44, 75, 76, 82a, 83, 87, and 108 (Kabat numbering as in Table 4), or residue 20, 87, 94, 95 and 112 (linear numbering as in Table 5).

[0049] In yet other embodiments, the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes at least one light or heavy chain immuglobulin or, more preferably, at least one light chain immuglobulin and at least one heavy chain immuglobulin. Preferably, the light chain immunoglobulin includes a modified non-human light chain variable region

comprising three CDRs from a non-human, e.g., murine, anti-PSMA light chain variable region (e.g., the murine J591 light chain variable region shown in SEQ ID NO:20 (see Figure 1B), or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126) and a modified light chain framework which differs from the framework of the non-human anti-PSMA light chain variable region, e.g., the murine J591 light chain variable region (SEQ ID NO:20 or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126), by at least one, two, three, four, five, six, seven, eight, nine, ten positions selected from the group consisting of:

a position within or adjacent to one or more of residues 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13, or a T cell epitope which includes one or more of residues 1-13 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, or a T cell epitope which includes one or more of residues residues 8-20 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29, or a T cell epitope which includes one or more of residues 17-29 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38 or 39, or a T cell epitope which includes one or more of residues 27-39 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 or 43, or a T cell epitope which includes one or more of residues 30-43 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, or 57, or a T cell epitope which includes one or more of residues 45- 57 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, or 68, or a T cell epitope which includes one or more of residues 56-68 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, or 83, or a T cell epitope which includes one or more of residues 71- 83 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84 or 85, or a T cell epitope which includes one or more of residues 73-85 (numbering as in Figure 3B); and

a position within or adjacent to one or more of residues 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, or 106, or a T cell epitope which includes one or more of residues 94-106 (numbering as in Figure 3B).

[0050] In yet other embodiments, the anti-PSMA antibody, or antigen-binding fragment thereof, includes at least one light or heavy chain immuglobulin or, more preferably, at least one light chain immuglobulin and at least one modified heavy chain immuglobulin. Preferably, the light chain immunoglobulin includes a modified non-human light chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA light chain variable region (e.g., the murine J415 light chain variable region shown in SEQ ID NO:48 (Figure 37), or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109) and a light chain framework which differs from the framework of the non-human anti-PSMA light chain variable region, e.g., the murine J415 light chain variable region (SEQ ID NO:48 or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109), by at least one, two, three, four, five, six, seven positions selected from the group consisting of:

a position within or adjacent to one or more of residues 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18, or a T cell epitope which includes one or more of residues 5-18 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24, or a T cell epitope which includes one or more of residues residues 11-24 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26, or a T cell epitope which includes one or more of residues 13-26 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, or a T cell epitope which includes one or more of residues 17-30 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or a T cell epitope which includes one or more of residues 27-40 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, or 44, or a T cell epitope which includes one or more of residues 31-44 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, or 69, or a T cell epitope which includes one or more of residues 56-69 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, or 73, or a T cell epitope which includes one or more of residues 60-73 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, or 83, or a T cell epitope which includes one or more of residues 70-83 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83 or 84, or a T cell epitope which includes one or more of residues 71-84 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85 or 86, or a T cell epitope which includes one or more of residues 73-86 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, or 92, or a T cell epitope which includes one or more of residues 76-92 (linear numbering as in Figure 6); and

a position within or adjacent to one or more of residues 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94, or a T cell epitope which includes one or more of residues 81-94 (linear numbering as in Figure 6).

[0051] In other embodiments, the heavy chain immunoglobulin of the anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human heavy chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA heavy chain variable region (e.g., the murine J591 heavy chain variable region shown in SEQ ID NO:19 (see Figure 1A), or the heavy chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126) and a heavy chain framework which differs from the framework of the non-human anti-PSMA heavy chain variable region (e.g., the murine J591 heavy chain variable region of SEQ ID NO:19 or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126), by at least one, two, three, five, seven, ten positions selected from the group consisting of:

a position within or adjacent to one or more of residues 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14, or a T cell epitope which includes one or more of residues 2-14 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22, or a T cell epitope which includes one or more of residues 10- 22 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28, or a T cell epitope which includes one or more of residues 16-28 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, or 42, or a T cell epitope which includes one or more of residues 30- 42 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, or 44, or a T cell epitope which includes one or more of residues 32-44 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, or 55, or a T cell epitope which includes one or more of residues 43-55 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, or 58, or a T cell epitope which includes one or more of residues 46-58 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70, or a T cell epitope which includes one or more of residues 58-70 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73 or 74, or a T cell epitope which includes one or more of residues 62-74 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80 or 81, or a T cell epitope which includes one or more of residues 70-81 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, or 93, or a T cell epitope which includes one or more of residues 81-93 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 95, or 96, or a T cell epitope which includes one or more of residues 84-96 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 91, 92, 93, 95, 96, 97, 98, 99, 100, 101, 102, or 103, or a T cell epitope which includes one or more of residues 91- 103 (numbering as in Figure 3A); and

a position within or adjacent to one or more of residues 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, or 112, or a T cell epitope which includes one or more of residues 100-112 (numbering as in Figure 3A).

[0052] In other embodiments, the heavy chain immunoglobulin of the anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human heavy chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA heavy chain variable region (e.g., the murine J415 heavy chain variable region shown in SEQ ID NO:47 (see Figure 5), or the heavy chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109) and a heavy chain framework which differs from the framework of the non-human anti-PSMA heavy chain variable region, e.g., the murine J591 heavy chain variable region of SEQ ID NO:47 or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109), by at least one, two, three, four, five positions selected from the group consisting of:

a position within or adjacent to one or more of residues 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23, or a T cell epitope which includes one or more of residues 10-23 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29, or a T cell epitope which includes one or more of residues 16-29 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34, or a T cell epitope which includes one or more of residues 21-34 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or a T cell epitope which includes one or more of residues 30- 43 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48, or a T cell epitope which includes one or more of residues 35-48 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, or 56, or a T cell epitope which includes one or more of residues 43-56 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58 or 59, or a T cell epitope which includes one or more of residues 46-59 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, or 62, or a T cell epitope which includes one or more of residues 49-62 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, or 77, or a T cell epitope which includes one or more of residues 64-77 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, or 93, or a T cell epitope which includes one or more of residues 80-93 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99, or a T cell epitope which includes one or more of residues 86-99 (numbering as in Figure 5); and

a position within or adjacent to one or more of residues 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, or 117, or a T cell epitope which includes one or more of residues 104-117 (numbering as in Figure 5).

[0053] In yet other embodiments, the anti-PSMA antibody, or antigen-binding fragment thereof, includes at least one light or heavy chain immuglobulin or, more preferably, at least one light chain immunoglobulin and at least one heavy chain immuglobulin. Preferably, the light chain immunoglobulin includes a non-human light chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA light chain variable region (e.g., the murine J591 light chain variable region shown in SEQ ID NO:20 (Figure 1B), or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126) and a light chain framework which differs from the framework of the non-human anti-PSMA light chain variable region, e.g., murine J591 light chain variable region, by at least one position while having a residue from the non-human anti-PSMA light chain variable region at at least one, two, three, five, seven, ten, fifteen, or twenty residues selected from the group consisting of 1, 2, 4-7, 12-19, 23, 31-41, 43-49, 57, 59, 61, 62, 64-75, 79, 82, 83, 85-87, 89, 98, 99, 101, 102, 105, and 106 (numbering as in Figure 3B). The light chain framework can differ at positions chosen from one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, nineteen, twenty or more residues selected from the group consisting of 3, 8, 9, 10, 11, 20, 21, 22, 42, 58, 60, 63, 76, 77, 78, 80, 83, 87, 100, 103, and 104 (numbering as in Figure 3B).

[0054] In yet other embodiments, the anti-PSMA antibody, or antigen-binding fragment thereof, includes at least one light or heavy chain immuglobulin or, more preferably, at least one light chain immunoglobulin and at least one heavy chain immuglobulin. Preferably, the modified light chain immunoglobulin includes a non-human light chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA light chain variable region (e.g., the murine J415 light chain variable region shown in SEQ ID NO:48 (Figure 6), or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109) and a light chain framework which differs from the framework of

the non-human anti-PSMA light chain variable region, e.g., murine J415 light chain variable region, by at least one position while having a residue from the non-human anti-PSMA light chain variable region at at least one, two, three, five, seven, ten, fifteen, or twenty residues selected from the group consisting of 1-12, 14, 16-18, 20-40, 42-62, 64-67, 69-79, and 81-107 (linear numbering as in Figure 6). The modified light chain framework can differ at at least one, two, three, four, five, six, or seven positions selected from the group consisting of 13, 15, 19, 41, 63, 68 and 80 (linear numbering as in Figure 6).

[0055] In other embodiments, the heavy chain immunoglobulin of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human heavy chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA heavy chain variable region (e.g., the murine J591 heavy chain variable region shown in SEQ ID NO:19 (Figure 1A), or the heavy chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126) and a modified heavy chain framework which differs from the framework of the non-human anti-PSMA heavy chain variable region by at least one position while having a residue from the non-human anti-PSMA heavy chain variable region at at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen residues selected from the group consisting of 1-4, 6-10, 13-15, 18, 20-25, 36-39, 42, 43, 45-49, 67-75, 78-83, 85, 86, 88-90, 92-98, 105-109, and 111-115 (numbering as in Figure 3A). The modified heavy chain framework can differ at at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen positions selected from the group consisting of 5, 11-12, 16-17, 19, 26-35, 40-41, 44, 50-66, 76-77, 84, 87, 91, 99-104, and 110 (numbering as in Figure 3A).

[0056] In other embodiments, the heavy chain immunoglobulin of the anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human heavy chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA heavy chain variable region (e.g., the murine J415 heavy chain variable region shown in SEQ ID NO:47 (Figure 5), or the heavy chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109) and a heavy chain framework which differs from the framework of the non-human anti-PSMA heavy chain variable region by at least one position while having a residue from the non-human anti-PSMA heavy chain variable region at at least one, two, three, four, or five residues selected from the group consisting of 1-19, 21-86, 88-93,

96-111, and 113-116 (numbering as in Figure 5). The heavy chain framework can differ at a positions selected from the group consisting of 20, 87, 94, 95 and 112 (numbering as in Figure 5).

[0057] In yet another aspect, the heavy chain immunoglobulin of the anti-PSMA antibody, or antigen-binding fragment thereof, includes a heavy chain variable region comprising at least one, two, three, four, five, six, seven, eight, nine, ten, twenty, twenty-five, thirty, thirty-five, forty, forty-five, or fifty amino acid residues chosen from one or more of the following residues and located at a position chosen from one or more of: residue 1 (glutamate), 2 (valine), 4 (leucine), 7 (serine), 8 (glycine), 11 (leucine), 14 (proline), 15 (glycine), 19 (lysine), 20 (isoleucine), 21 (serine), 22 (cysteine), 25 (serine), 26 (glycine), 28 (threonine), 29 (phenylalanine), 32 (tyrosine), 36 (tryptophan), 37 (valine), 38 (arginine/lysine), 39 (glutamine), 41 (proline), 43 (lysine), 44 (glycine), 45 (leucine), 46 (glutamate), 47 (tryptophan), 51 (isoleucine), 67 (arginine/lysine), 73 (aspartate), 75 (serine), 80 (tyrosine), 85 (serine), 86 (leucine), 87 (arginine), 89 (glutamate), 90 (aspartate), 91 (threonine), 92 (alanine), 93 (valine), 94 (tyrosine), 95 (tyrosine), 96 (cysteine), 100 (tryptophan), 101 (asparagine), 105 (tryptophan), 106 (glycine), 107 (glutamine), 108 (glycine), 109 (threonine), 112 (threonine), 113 (valine), 114 (serine), or 115 (serine) (linear numbering as shown in Figure 3A).

[0058] In one embodiment, the heavy chain immunoglobulin of the anti-PSMA antibody, or antigen-binding fragment thereof, includes one or more of:

a framework region 1 having at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen amino acids selected from the group consisting of residue 1 (glutamate), 2 (valine), 4 (leucine), 7 (serine), 8 (glycine), 11 (leucine), 14 (proline), 15 (glycine), 19 (lysine), 20 (isoleucine), 21 (serine), 22 (cysteine), and 25 (serine) (linear numbering as shown in Figure 3A);

a CDR1 having at least one, two, three, four amino acids selected from the group consisting of residue 26 (glycine), 28 (threonine), 29 (phenylalanine), and 32 (tyrosine) (linear numbering as shown in Figure 3A);

a framework region 2 having at least one, two, three, four, five, six, seven, eight, nine, ten amino acids selected from the group consisting of residue 36 (tryptophan), 37 (valine), 38 (arginine/lysine), 39 (glutamine), 41 (proline), 43 (lysine), 44 (glycine), 45 (leucine), 46 (glutamate), and 47 (tryptophan) (linear numbering as shown in Figure 3A);

a CDR2 having at least one isoleucine at position 51 (linear numbering as shown in Figure 3A);

a framework region 3 having at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen amino acids selected from the group consisting of residue 67 (arginine/lysine), 73 (aspartate), 75 (serine), 80 (tyrosine), 85 (serine), 86 (leucine), 87 (arginine), 89 (glutamate), 90 (aspartate), 91 (threonine), 92 (alanine), 93 (valine), 94 (tyrosine), 95 (tyrosine), and 96 (cysteine) (linear numbering as shown in Figure 3A);

a CDR3 having at least one, two amino acids selected from the group consisting of residue 100 (tryptophan) and 101 (asparagine) (linear numbering as shown in Figure 3A); or

a framework region 4 having at least one, two, three, four, five, six, seven, eight, nine amino acids selected from the group consisting of residue 105 (tryptophan), 106 (glycine), 107 (glutamine), 108 (glycine), 109 (threonine), 112 (threonine), 113 (valine), 114 (serine), and 115 (serine) (linear numbering as shown in Figure 3A).

[0059] In yet another embodiment, the light chain immunoglobulin of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes a light chain variable region comprising at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, twenty, thirty, forty, fifty, sixty, or seventy amino acids chosen from one or more of the following residues and located at a position chosen from one or more of: residue 2 (isoleucine), 4 (methionine), 5 (threonine), 6 (glutamine), 8 (proline), 10 (serine), 12 (serine), 14 (serine), 16 (glycine), 17 (glutamate/aspartate), 18 (arginine), 20 (threonine), 21 (leucine), 22 (threonine), 23 (cysteine), 24 (lysine), 25 (alanine), 26 (serine), 29 (valine), 30 (glycine), 31 (threonine), 33 (valine), 35 (tryptophan), 36 (tryrosine), 37 (glutamine), 38 (glutamine), 39 (lysine), 40 (proline), 43 (serine), 44 (proline), 45 (lysine), 47 (leucine), 48 (isoleucine), 49 (tyrosine), 51 (alanine), 52 (serine), 54 (arginine), 56 (threonine), 57 (glycine), 59 (proline), 61 (arginine), 62 (phenylalanine), 63 (serine), 64 (glycine), 65 (serine), 66 (glycine), 67 (serine), 68 (glycine), 69 (threonine), 70 (aspartate), 71 (phenylalanine), 73 (leucine), 74 (threonine), 75 (threonine), 76 (serine), 77 (serine), 79 (glutamine), 81 (glutamate), 82 (aspartate), 85 (aspartate), 86 (tyrosine), 87 (tyrosine), 88 (cysteine), 90 (glutamine), 95 (proline), 97 (threonine), 98 (phenylalanine), 99 (glycine), 101 (glycine), 102 (threonine), 103 (lysine), 105 (glutamate/aspartate), or 107 (lysine) (linear numbering as in Figure 3B).

[0060] In one embodiment, the light chain immunoglobulin of the anti-PSMA antibody, or antigen-binding fragment thereof, includes one or more of:

a framework region 1 having at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, fourteen, fifteen amino acids selected from the group consisting of residue 2 (isoleucine), 4 (methionine), 5 (threonine), 6 (glutamine), 8 (proline), 10 (serine), 12 (serine), 14 (serine), 16 (glycine), 17 (glutamate/aspartate), 18 (arginine), 20 (threonine), 21 (leucine), 22 (threonine), and 23 (cysteine) (linear numbering as shown in Figure 3B);

a CDR1 having at least one, two, three, four, five, six, seven amino acids selected from the group consisting of residue 24 (lysine), 25 (alanine), 26 (serine), 29 (valine), 30 (glycine), 31 (threonine), and 33 (valine) (linear numbering as shown in Figure 3B);

a framework region 2 having at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve amino acids selected from the group consisting of residue 35 (tryptophan), 36 (tryrosine), 37 (glutamine), 38 (glutamine), 39 (lysine), 40 (proline), 43 (serine), 44 (proline), 45 (lysine), 47 (leucine), 48 (isoleucine), and 49 (tyrosine) (linear numbering as shown in Figure 3B);

a CDR2 having at least one, two, three, four amino acids selected from the group consisting of residue 51 (alanine), 52 (serine), 54 (arginine), and 56 (threonine) (linear numbering as shown in Figure 3B);

a framework region 3 having at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four amino acids selected from the group consisting of residue 59 (proline), 61 (arginine), 62 (phenylalanine), 63 (serine), 64 (glycine), 65 (serine), 66 (glycine), 67 (serine), 68 (glycine), 69 (threonine), 70 (aspartate), 71 (phenylalanine), 73 (leucine), 74 (threonine), 75 (threonine), 76 (serine), 77 (serine), 79 (glutamine), 81 (glutamate), 82 (aspartate), 85 (aspartate), 86 (tyrosine), 87 (tyrosine), and 88 (cysteine) (linear numbering as shown in Figure 3B);

a CDR3 having at least one, two, three, four amino acids selected from the group consisting of residue 90 (glutamine), 95 (proline), 97 (threonine), and 98 (phenylalanine) (linear numbering as shown in Figure 3B); or

a framework region 4 having at least one, two, three, four, five, six amino acid selected from the group consisting of residue 99 (glycine), 101 (glycine), 102 (threonine), 103 (lysine), 105 (glutamate/aspartate), and 107 (lysine) (linear numbering as shown in Figure 3B).

[0061] An anti-PSMA antibody, e.g., a modified anti-PSMA antibody, or antigen-binding fragment thereof, described herein can be used alone, e.g., can be administered to a subject, or used *in vitro*, in non-derivatized or unconjugated forms. In other embodiments, the anti-PSMA antibody, or antigen-binding fragment thereof, can be derivatized or linked to another molecular entity, typically a label or a therapeutic (e.g., a cytotoxic or cytostatic) agent. The molecular entity can be, e.g., another peptide, protein (including, e.g., a viral coat protein of, e.g., a recombinant viral particle), a non-peptide chemical compound, isotope, etc. The anti-PSMA antibody, or antigen-binding fragment thereof, can be functionally linked, e.g., by chemical coupling, genetic fusion, non-covalent association or otherwise, to one or more other molecular entities. For example, the anti-PSMA antibody, or antigen-binding fragment thereof, can be coupled to a label, such as a fluorescent label, a biologically active enzyme label, a radioisotope (e.g., a radioactive ion), a nuclear magnetic resonance active label, a luminescent label, or a chromophore. In other embodiments, the anti-PSMA antibody, or antigen-binding fragment thereof, can be coupled to a therapeutic agent, e.g., a cytotoxic moiety, e.g., a therapeutic drug, a radioisotope, molecules of plant, fungal, or bacterial origin, or biological proteins (e.g., protein toxins) or particles (e.g., recombinant viral particles, e.g., via a viral coat protein), or mixtures thereof. The therapeutic agent can be an intracellularly active drug or other agent, such as short-range radiation emitters, including, for example, short-range, high-energy α-emitters, as described herein. In some preferred embodiments, the anti-PSMA antibody, or antigen binding fragment thereof, can be coupled to a molecule of plant or bacterial origin (or derivative thereof), e.g., a maytansinoid (e.g., maytansinol or the DM1 maytansinoid, see Fig. 15), a taxane, or a calicheamicin. A radioisotope can be an α-, β-, or γ-emitter, or an β- and γ-emitter. Radioisotopes useful as therapeutic agents include yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), praseodymium, astatine (^{211}At), rhenium (^{186}Re), bismuth (^{212}Bi or ^{213}Bi), and rhodium (^{188}Rh). Radioisotopes useful as labels, e.g., for use in diagnostics, include iodine (^{131}I or ^{125}I), indium (^{111}In), technetium (^{99}mTc), phosphorus (^{32}P), carbon (^{14}C), and tritium (^{3}H), or one of the therapeutic isotopes listed above. The anti-PSMA antibody, or antigen-binding fragment

thereof can also be linked to another antibody to form, e.g., a bispecific or a multispecific antibody.

[0062] In another aspect, the invention features, an anti-PSMA antibody, e.g., an antibody described herein, coupled, e.g., by covalent linkage, to a proteosome inhibitor or a topoisomerase inhibitor. [(1R)-3-methyl-1-[[(2S)-1-oxo-3-phenyl-2-[(3-mercaptoacetyl) amino]propyl]amino]butyl] Boronic acid is a suitable proteosome inhibitor. N,N'-bis[2-(9-methylphenazine-1-carboxamido)ethyl]-1,2-ethanediamine is a sutiable topoisomerase inhibitor.

[0063] In another aspect, the invention provides, compositions, e.g., pharmaceutical compositions, which include a pharmaceutically acceptable carrier, excipient or stabilizer, and at least one of the anti-PSMA antibodies, e.g., the modified anti-PSMA antibodies (or fragments thereof) described herein. In a preferred embodiment the antibody is conjugated to a label or a therapeutic agent. In one embodiment, the compositions, e.g., the pharmaceutical compositions, comprise a combination of two or more of the aforesaid anti-PSMA antibodies. For example, a composition, e.g., pharmaceutical composition, which comprises a deimmunized J591 antibody, in combination with another anti-PSMA antibody, or an antibody to another tumor cell-associated antigen, e.g., EGF receptor, Her-2/neu, etc. Combinations of the anti-PSMA antibody and a drug, e.g., a therapeutic agent (e.g., a cytototoxic or cytostatic drug, e.g., DM1, calicheamicin, or taxanes, topoisomerase inhibitors, or an immunomodulatory agent, e.g., IL-1, 2, 4, 6, or 12, interferon alpha or gamma, or immune cell growth factors such as GM-CSF) are also within the scope of the invention.

[0064] The invention also features nucleic acid sequences that encode a heavy and light chain immunoglobulin described herein. For example, the invention features, a first and second nucleic acid encoding a modified heavy and light chain variable region, respectively, of a modified anti-PSMA antibody molecule as described herein. In another aspect, the invention features host cells and vectors containing the nucleic acids of the invention.

[0065] In another aspect, the invention features a method of producing an anti-PSMA antibody, e.g., a modified anti-PSMA antibody, or antigen-binding fragment thereof. The method includes:

providing a first nucleic acid encoding a heavy chain variable region, e.g., a modified heavy chain variable region as described herein;

providing a second nucleic acid encoding a light chain variable region, e.g., a modified light chain variable region as described herein; and

introducing said first and second nucleic acids into a host cell under conditions that allow expression and assembly of said light and heavy chain variable regions.

[0066] The first and second nucleic acids can be linked or unlinked, e.g., expressed on the same or different vector, respectively.

[0067] The host cell can be a eukaryotic cell, e.g., a mammalian cell, an insect cell, a yeast cell, or a prokaryotic cell, e.g., *E. coli*. For example, the mammalian cell can be a cultured cell or a cell line. Exemplary mammalian cells include lymphocytic cell lines (e.g., NS0), Chinese hamster ovary cells (CHO), COS cells, oocyte cells, and cells from a transgenic animal, e.g., mammary epithelial cell. For example, nucleic acids encoding the modified antibody described herein can be expressed in a transgenic animal. In one embodiment, the nucleic acids are placed under the control of a tissue-specific promoter (e.g., a mammary specific promoter) and the antibody is produced in the transgenic animal. For example, the antibody molecule is secreted into the milk of the transgenic animal, such as a transgenic cow, pig, horse, sheep, goat or rodent.

[0068] The invention also features a method of ablating or killing, a cell, e.g., a prostatic cell (e.g., a cancerous or non-cancerous prostatic cell, e.g., a normal, benign or hyperplastic prostatic epithelial cell), or a malignant, non-prostatic cell, e.g., cell found in a non-prostatic solid tumor that, e.g., has vasculature which expresses PSMA, a soft tissue tumor, or a metastatic lesion (e.g., a cell found in renal, urothelial (e.g. bladder), colonic, rectal, pulmonary, breast or hepatic cancers and/or metastases thereof). Methods of the invention include contacting the cell, or a nearby cell, e.g., a vascular endothelial cell proximate to the cell, with an anti-PSMA antibody as described herein, e.g., a modified anti-PSMA antibody, in an amount sufficient to ablate or kill, the cell. Alternatively, an anti-PSMA antibody as described herein, e.g., a modified anti-PSMA antibody, preferably a fragment of a modified anti-PSMA antibody, can be conjugated to a viral particle, e.g., to a coat protein of a viral particle. The anti-PSMA/viral particle conjugate can be used to target prostate cells, e.g., cancerous prostate cells, with genetically engineered viral particles that infect the cells and express, e.g., pro apoptotic genes, to thereby kill the cells or inhibit cell growth.

[0069] The methods can be used on cells in culture, e.g. *in vitro* or *ex vivo*. For example, prostatic cells (e.g., malignant or normal, benign or hyperplastic prostate epithelial cells) or non-prostatic cancerous or metastatic cells (e.g., renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), pancreatic (e.g., pancreatic duct), melanoma (e.g., malignant melanoma), or soft tissue sarcoma cancerous cells) can be cultured *in vitro* in culture medium and the contacting step can be effected by adding the modified anti-PSMA antibody or fragment thereof, to the culture medium. The method can be performed on cells (e.g., prostatic cells, or non-prostatic cancerous or metastatic cells) present in a subject, as part of an *in vivo* (e.g., therapeutic or prophylactic) protocol.

[0070] Methods of the invention can be used, for example, to treat or prevent a disorder, e.g., a prostatic disorder (e.g., a cancerous or non-cancerous prostatic disorder, e.g., a benign or hyperplastic prostatic disorder), or a non-prostatic disorder (e.g., cancer, e.g., malignant cancer), by administering to a subject an antibody described herein, preferably a modified PSMA antibody, or antigen-binding fragment thereof, in an amount effective to treat or prevent such disorder. Particularly preferred antibodies include modified antibodies having CDRs from any of a J591, J415, J533 or E99, and in particular deimmunized versions of these antibodies, particularly deJ591 or deJ415. Examples of prostatic disorders that can be treated or prevented include, but are not limited to, genitourinary inflammation (e.g., inflammation of smooth muscle cells) as in prostatitis; benign enlargement, for example, nodular hyperplasia (benign prostatic hypertrophy or hyperplasia); and cancer, e.g., adenocarcinoma or carcinoma, of the prostate and/or testicular tumors. Methods and compositions disclosed herein are particularly useful for treating metastatic lesions associated with prostate cancer. In some embodiments, the patient will have undergone one or more of prostatectomy, chemotherapy, or other anti-tumor therapy and the primary or sole target will be metastatic lesions, e.g, metastases in the bone marrow or lymph nodes. Examples of non-prostatic cancerous disorders include, but are not limited to, solid tumors, soft tissue tumors, and particularly metastatic lesions. Examples of solid tumors include malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting lung, breast, lymphoid, gastrointestinal (e.g., colon), genitals and genitourinary tract (e.g., renal, urothelial, bladder cells), pharynx, CNS (e.g., neural or glial cells), skin (e.g., melanoma), and pancreas, as well as adenocarcinomas which include

malignancies such as most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. Methods and compositions disclosed herein are particularly useful for treating metastatic lesions associated with the aforementioned cancers. In some embodiments, the patient will have undergone one or more of surgical removal of a tissue, chemotherapy, or other anti-cancer therapy and the primary or sole target will be metastatic lesions, e.g, metastases in the bone marrow or lymph nodes.

[0071] In a preferred embodiment the subject is treated to prevent a disorder, e.g., a prostatic disorder. The subject can be one at risk for the disorder, e.g., a subject having a relative afflicted with the disorder, e.g., a subject with one or more of a grandparent, parent, uncle or aunt, sibling, or child who has or had the disorder, or a subject having a genetic trait associated with risk for the disorder. In a preferred embodiment the disorder is a prostatic disorder (e.g., a cancerous or non-cancerous prostatic disorder, e.g., a benign or hyperplastic prostatic disorder), or a non-prostatic disorder (e.g., cancer, e.g., malignant cancer) and the subject has one or more of a grandfather, father, uncle, brother, or son who has or had the disorder, or a subject having a genetic trait associated with risk for the disorder.

[0072] The subject can be a mammal, e.g., a primate, preferably a higher primate, e.g., a human (e.g., a patient having, or at risk of, a disorder described herein, e.g., a prostatic or a cancer disorder). In one embodiment, the subject is a patient having prostate cancer (e.g., a patient suffering from recurrent or metastatic prostate cancer).

[0073] The modified anti-PSMA antibody or fragment thereof, e.g., a modified anti-PSMA antibody or fragment thereof as described herein, can be administered to the subject systemically (e.g., orally, parenterally, subcutaneously, intravenously, rectally, intramuscularly, intraperitoneally, intranasally, transdermally, or by inhalation or intracavitary installation), topically, or by application to mucous membranes, such as the nose, throat and bronchial tubes.

[0074] The methods of the invention, e.g., methods of treatment or preventing, can further include the step of monitoring the subject, e.g., for a change (e.g., an increase or decrease) in one or more of: tumor size; levels of a cancer marker, e.g., levels of PSA, alkaline phosphatase, or serum hemoglobin for a patient with prostate cancer; the rate of appearance of new lesions, e.g., in a bone scan; the appearance of new disease-related symptoms; the size of soft tissue mass, e.g., a decreased or stabilization; quality of life, e.g., amount of disease

associated pain, e.g., bone pain; or any other parameter related to clinical outcome. The subject can be monitored in one or more of the following periods: prior to beginning of treatment; during the treatment; or after one or more elements of the treatment have been administered. Monitoring can be used to evaluate the need for further treatment with the same modified anti-PSMA antibody or fragment thereof or for additional treatment with additional agents. Generally, a decrease in one or more of the parameters described above is indicative of the improved condition of the subject, although with serum hemoglobin levels, an increase can be associated with the improved condition of the subject.

[0075] The methods of the invention can further include the step of analyzing a nucleic acid or protein from the subject, e.g., analyzing the genotype of the subject. In one embodiment, a nucleic acid encoding human PSMA and/or an upstream or downstream component(s) of human PSMA signalling, e.g., an extracellular or intracellular activator or inhibitor of human PSMA, is analyzed. The analysis can be used, e.g., to evaluate the suitability of, or to choose between alternative treatments, e.g., a particular dosage, mode of delivery, time of delivery, inclusion of adjunctive therapy, e.g., administration in combination with a second agent, or generally to determine the subject's probable drug response phenotype or genotype. The nucleic acid or protein can be analyzed at any stage of treatment, but preferably, prior to administration of the modified anti-PSMA antibody or fragment thereof to thereby determine appropriate dosage(s) and treatment regimen(s) of the modified anti-PSMA antibody or fragment thereof (e.g., amount per treatment or frequency of treatments) for prophylactic or therapeutic treatment of the subject.

[0076] The modified anti-PSMA antibody or fragment thereof can be used alone in unconjugated form to thereby ablate or kill the PSMA-expressing prostatic or cancerous cells by, e.g., antibody-dependent cell killing mechanisms such as complement-mediated cell lysis and/or effector cell-mediated cell killing. In other embodiments, the modified anti-PSMA antibody or fragment thereof can be bound to a substance, e.g., a cytotoxic agent or moiety, e.g., a therapeutic drug, a compound emitting radiation, molecules of plant, fungal, or bacterial origin, or a biological protein (e.g., a protein toxin) or particle (e.g., a recombinant viral particle, e.g., via a viral coat protein). For example, the modified anti-PSMA antibody, or antigen-binding fragment thereof, can be coupled to a radioactive isotope such as an α-, β-, or γ-emitter, or a β- and γ-emitter. Examples of radioactive isotopes include iodine (^{131}I or ^{125}I), yttrium (^{90}Y),

lutetium (^{177}Lu), actinium (^{225}Ac), praseodymium, or bismuth (^{212}Bi or ^{213}Bi). Alternatively, the anti-PSMA antibody, or antigen-binding fragment thereof, can be coupled to a biological protein, a molecule of plant or bacterial origin (or derivative thereof), e.g., a maytansinoid (e.g., maytansinol or DM1), as well as a taxane (e.g, taxol or taxotere), or calicheamicin. The maytansinoid can be, for example, maytansinol or a maytansinol analogue. Examples of maytansinol analogues include those having a modified aromatic ring (e.g., C-19-decloro, C-20-demethoxy, C-20-acyloxy) and those having modifications at other positions (e.g., C-9-CH, C-14-alkoxymethyl, C-14-hydroxymethyl or aceloxymethyl, C-15-hydroxy/acyloxy, C-15-methoxy, C-18-N-demethyl, 4,5-deoxy). Maytansinol and maytansinol analogues are described, for example, in U.S. Patent Number 6,333,410, the contents of which is incorporated herein by reference. The calicheamicin can be, for example, a bromo-complex calicheamicin (e.g., an alpha, beta or gamma bromo-complex), an iodo-complex calicheamicin (e.g., an alpha, beta or gamma iodo-complex), or analogs and mimics thereof. Bromo-complex calicheamicins include α_1-BR, α_2-BR, α_3-BR, α_4-BR, β_1-BR, β_2-BR and γ_1-BR. Iodo-complex calicheamicins include α_1-I, α_2-I, α_3-I, β_1-I, β_2-I, δ_1-I and γ_1-BR. Calicheamicin and mutants, analogs and mimics thereof are described, for example, in U.S. Patent Numbers 4,970,198, issued November 13, 1990, 5,264,586, issued November 23, 1993, 5,550,246, issued August 27, 1996, 5,712,374, issued January 27, 1998, and 5,714,586, issued February 3, 1998, the contents of which are incorporated herein by reference. Maytansinol can be coupled to antibodies using, e.g., an N-succinimidyl 3-(2-pyridyldithio)proprionate (also known as N-succinimidyl 4-(2-pyridyldithio)pentanoate or SPP), 4-succinimidyl-oxycarbonyl-a-(2-pyridyldithio)-toluene (SMPT), N-succinimidyl-3-(2-pyridyldithio)butyrate (SDPB), 2-iminothiolane, or S-acetylsuccinic anhydride.

[0077] The methods and compositions of the invention can be used in combination with other therapeutic modalities. In one embodiment, the methods of the invention include administering to the subject a modified anti-PSMA antibody or fragment thereof, e.g., a modified anti-PSMA antibody or fragment thereof as described herein, in combination with a cytotoxic agent, in an amount effective to treat or prevent said disorder. The antibody and the cytotoxic agent can be administered simultaneously or sequentially. In other embodiments, the methods and compositions of the invention are used in combination with surgical and/or radiation procedures. In yet other embodiments, the methods can be used in combination with

immunodulatory agents, e.g., IL-1, 2, 4, 6, or 12, or interferon alpha or gamma, or immune cell growth factors such as GM-CSF.

[0078] Exemplary cytotoxic agents that can be administered in combination with the anti-PSMA antibodies include antimicrotubule agents, topoisomerase inhibitors, antimetabolites, mitotic inhibitors, alkylating agents, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis and radiation.

[0079] In therapies of prostatic disorders, e.g., prostate cancer, the anti-PSMA antibodies can be used in combination with existing therapeutic modalities, e.g., prostatectomy (partial or radical), radiation therapy, hormonal therapy, androgen ablation therapy, and cytotoxic chemotherapy. Typically, hormonal therapy works to reduce the levels of androgens in a patient, and can involve administering a leuteinizing hormone-releasing hormone (LHRH) analog or agonist (e.g., Lupron, Zoladex, leuprolide, buserelin, or goserelin), as well as antagonists (e.g., Abarelix). Non-steroidal anti-androgens, e.g., flutamide, bicalutimade, or nilutamide, can also be used in hormonal therapy, as well as steroidal anti-androgens (e.g., cyproterone acetate or megastrol acetate), estrogens (e.g., diethylstilbestrol), surgical castration, PROSCAR™, secondary or tertiary hormonal manipulations (e.g., involving corticosteroids (e.g., hydrocortisone, prednisone, or dexamethasone), ketoconazole, and/or aminogluthethimide), inhibitors of 5a-reductase (e.g., finisteride), herbal preparations (e.g., PC-SPES), hypophysectomy, and adrenalectomy. Furthermore, hormonal therapy can be performed intermittently or using combinations of any of the above treatments, e.g., combinded use of leuprolide and flutamide.

[0080] Any combination and sequence of modified anti-PSMA and other therapeutic modalities can be used. The modified anti-PSMA and other therapeutic modalities can be administered during periods of active disorder, or during a period of remission or less active disease. The modified anti-PSMA and other therapeutic modalities can be administered before treatment, concurrently with treatment, posttreatment, or during remission of the disorder.

[0081] In another aspect, the invention features methods for detecting the presence of a PSMA protein in a sample *in vitro* (e.g., a biological sample, e.g., serum, semen or urine, or a tissue biopsy, e.g., from a prostatic or cancerous lesion). The subject method can be used to evaluate, e.g., diagnose or stage a disorder described herein, e.g., a prostatic or cancerous disorder. The method includes: (i) contacting the sample (and optionally, a reference, e.g., a

control sample) with a modified anti-PSMA antibody, or fragment thereof, as described herein, under conditions that allow interaction of the anti-PSMA antibody and the PSMA protein to occur; and (ii) detecting formation of a complex between the anti-PSMA antibody, and the sample (and optionally, the reference, e.g., control, sample). Formation of the complex is indicative of the presence of PSMA protein, and can indicate the suitability or need for a treatment described herein. For example, a statistically significant change in the formation of the complex in the sample relative to the reference sample, e.g., the control sample, is indicative of the presence of PSMA in the sample. In some embodiments, the methods can include the use of more than one anti-PSMA antibody, e.g., two anti-PSMA antibodies that bind to different epitopes on PSMA. For example, the method can involve an ELISA assay, e.g., as described in Example 19.

[0082] In yet another aspect, the invention provides a method for detecting the presence of PSMA *in vivo* (e.g., *in vivo* imaging in a subject). The method can be used to evaluate, e.g., diagnose or stage a disorder described herein, e.g., a prostatic or a cancerous disorder, in a subject, e.g., a mammal, e.g., a primate, e.g., a human. The method includes: (i) administering to a subject a modified anti-PSMA antibody (or antigen binding fragment thereof), under conditions that allow interaction of the modified anti-PSMA antibody (or fragment thereof) and the PSMA protein to occur; and (ii) detecting formation of a complex between the antibody or fragment and PSMA. A statistically significant change in the formation of the complex in the subject relative to the reference, e.g., the control subject or subject's baseline, is indicative of the presence of the PSMA.

[0083] In other embodiments, a method of diagnosing or staging a disorder as described herein (e.g., a prostatic or cancerous disorder) is provided. The method includes: (i) identifying a subject having, or at risk of having, the disorder; (ii) obtaining a sample of a tissue or cell affected with the disorder; (iii) contacting said sample or a control sample with an anti-PSMA antibody as described herein, e.g., a modified anti-PSMA antibody or fragment, under conditions that allow interaction of the binding agent and the PSMA protein to occur, and (iv) detecting formation of a complex. A statistically significant increase in the formation of the complex between the antibody (or fragment thereof) with respect to a reference sample, e.g., a control sample, is indicative of the disorder or the stage of the disorder.

[0084] Preferably, the modified anti-PSMA antibody or fragment thereof, used in the *in vivo* and *in vitro* diagnostic methods is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound binding agent. Suitable detectable substances include various biologically active enzymes, prosthetic groups, fluorescent materials, luminescent materials, paramagnetic (e.g., nuclear magnetic resonance active) materials, and radioactive materials. In some embodiments, the modified anti-PSMA antibody or fragment thereof is coupled to a radioactive ion, e.g., indium (^{111}In), iodine (^{131}I or ^{125}I), yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), bismuth (^{212}Bi or ^{213}Bi), sulfur (^{35}S), carbon (^{14}C), tritium (^{3}H), rhodium (^{188}Rh), technetium (^{99}mTc), praseodymium, or phosphorous (^{32}P).

[0085] In another aspect, the invention provides a method for determining the dose, e.g., radiation dose, that different tissues are exposed to when a subject, e.g., a human subject, is administered an anti-PSMA antibody that is conjugated to a radioactive isotope. The method includes: (i) administering an anti-PSMA antibody as described herein, e.g., a modified anti-PSMA antibody, that is labeled with a radioactive isotope, e.g., ^{111}In, to a subject; (ii) measuring the amount of radioactive isotope located in different tissues, e.g., prostate, liver, kidney, or blood, at various time points until most, e.g., 50%, 80%, 90%, 95%, or more, of the radioactive isotope has been eliminated from the body of the subject; and (iii) caluculating the total dose of radiation received by each tissue analyzed. In some embodiements, the measurements are taken at scheduled time points, e.g., day 1, 2, 3, 5, 7, and 12, following administration (at day 0) of the radioactively labeled anti-PSMA antibody to the subject. In some embodiments, the radiation dose that a tissue receives for one radioactive isotope, e.g., a gamma-emitter, e.g., ^{111}In, can be used to calculate the expected dose that the same tissue would receive from a different radioactive isotope, e.g., a beta-emitter, e.g., ^{90}Y.

[0086] In another aspect, the invention features methods of treating pain, e.g., reducing pain, experienced by a subject having or diagnosed with prostate disease, e.g., benign prostatic hyperplasia or prostate cancer, or non-prostate cancer, e.g., a cancer having vasculature which expresses PSMA (e.g., renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), or pancreatic (e.g., pancreatic duct) cancer, melanoma (e.g., malignant melanoma), or soft tissue sarcoma). The methods include administering an anti-PSMA antibody as described herein, e.g., a modified anti-PSMA antibody, to a subject in an amount sufficient to treat, e.g., reduce, the

pain associated with prostate disease or non-prostate cancer. In some embodiments, the subject may have no signs of prostate disease or non-prostate cancer other than, e.g., elevated levels of serum PSA and the sensation of pain. The pain can be bone pain, as well as, pain associated with obstructive voiding symptoms due to enlarged prostate, e.g., urinary hesitancy or diminished urinary stream, frequency or nocturia. The treatment of pain using the modified anti-PSMA antibodies of the invention can lead to a decreased or dramatically lowered need, or even eliminate the need, for analgesics, e.g., narcotics. In addition, by reducing pain, the methods of treatment can restore the mobility of, e.g., limbs, that have become dysfunctional as a result of pain associated with movement.

[0087] In some embodiments, the modified anti-PSMA antibody is administered in an unconjugated form in an amount sufficient to treat, e.g., reduce, pain associated with prostate disease or non-prostate cancer. In other embodiments, the modified anti-PSMA antibody, or antigen-binding fragment thereof, is administered in a derivatized form, e.g., linked to another functional molecule, as described herein.

[0088] Other features and advantages of the instant invention will become more apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0089] *Figures 1A-1B* depict the amino acid sequence of murine J591 heavy and light chain variable region, respectively. The location of the CDRs is indicated in the Figures; the amino acid numbering is according the Kabat numbering (see, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Note that the CDRs are considered to encompass the Chothia loops and the Kabat hypervariable regions together and the sequences have been annotated accordingly. Heavy Chain: CDR1 is depicted in SEQ ID NO:1; CDR2 is depicted in SEQ ID NO:2; CDR3 is depicted in SEQ ID NO:3; the framework excluding CDR regions is depicted in SEQ ID NO:7; and the framework including CDR regions is depicted in SEQ ID NO:19. Light Chain: CDR1 is depicted in SEQ ID NO:4; CDR2 is depicted in SEQ ID NO:5; CDR3 is depicted in SEQ ID NO:6; the framework excluding CDR regions is depicted in SEQ ID NO:8; and the framework including CDR regions is depicted in SEQ ID NO:20.

[0090] *Figures 2A-2B* depict the amino acid sequence of the deimmunized J591 heavy and light chain variable region, respectively. The location of the CDRs is indicated in the Figures; the amino acid numbering is according the Kabat numbering (see, Kabat, E.A., *et al.* (1991) *supra*). Note that the CDRs are considered to encompass the Chothia loops and the Kabat hypervariable regions together and the sequences have been annotated accordingly. Heavy Chain: CDR1 is depicted in SEQ ID NO:1; CDR2 is depicted in SEQ ID NO:2; CDR3 is depicted in SEQ ID NO:3; framework 1 is depicted in SEQ ID NO:9; framework 2 is depicted in SEQ ID NO:10; framework 3 is depicted in SEQ ID NO:11; framework 4 is depicted in SEQ ID NO:12; the framework excluding CDR regions is depicted in SEQ ID NO:17; and the framework including CDR regions is depicted in SEQ ID NO:21. Light Chain: CDR1 is depicted in SEQ ID NO:4; CDR2 is depicted in SEQ ID NO:5; CDR3 is depicted in SEQ ID NO:6; framework 1 is depicted in SEQ ID NO:13; framework 2 is depicted in SEQ ID NO:14; framework 3 is depicted in SEQ ID NO:15; framework 4 is depicted in SEQ ID NO:16; the framework excluding CDR regions is depicted in SEQ ID NO:18; and the framework including CDR regions is depicted in SEQ ID NO:22.

[0091] *Figures 3A-3B* depict an alignment of the murine J591 and deimmunized heavy chain variable regions (3A; SEQ ID NO:19 and 21, respectively) and light chain variable regions (3B; SEQ ID NO:20 and 22, respectively). Potential T cell epitopes (identified using a peptide threading program) in murine J591 VH and VK are shown in Figures 3A-3B, respectively. The 13-mer peptides predicted to bind to MHC class II are indicated by the underline; the CDRs are located at residues 26 to 35, 50 to 66, and 99-104 of Figure 3A and residues 24 to 34, 50 to 56, and 89 to 97 of Figure 3B; and residues altered in the deimmunized heavy and light chain variable regions are boxed. Where possible, amino acid substitutions are those commonly used in human germline VH regions. The amino acid numbering is linear, not according to Kabat.

[0092] *Figures 4A-4B* depict the nucleotide sequences of the deimmunized J591 heavy and light chain variable region, respectively. Figure 4A shows an alignment of the coding and noncoding nucleotide strands of deimmunized J591 heavy chain variable region (SEQ ID NOs:23 and 24, respectively) with the corresponding amino acid sequence (SEQ ID NO:27). Figure 4B shows an alignment of the coding and noncoding nucleotide strands of deimmunized J591 light chain variable region (SEQ ID NOs:25 and 26, respectively) with the corresponding

amino acid sequence (SEQ ID NO:28). The location of the signal peptide and CDRs 1-3 is indicated in each alignment.

[0093] *Figure 5* depicts an alignment of the amino acid sequences for the murine and several deimmunized heavy chain variable regions of the J415 antibody. The murine amino acid sequence is shown as J415VH (SEQ ID NO:47); the deimmunized sequences are depicted as J415DIVH1 (amino acid residues 18 to 133 of SEQ ID NO:54), J415DIVH2 (SEQ ID NO:59), J415DIVH3 (SEQ ID NO:60), and J415DIVH4 (SEQ ID NO:49). The preferred sequence is J415DIVH4 (SEQ ID NO:49). The amino acid replacements are indicated by the boxed residues. A consensus sequence is labeled "majority" (SEQ ID NO:61).

[0094] *Figure 6* depicts an alignment of the amino acid sequences for the murine and several deimmunized light chain variable regions of the J415 antibody. The murine amino acid sequence is shown as J415VK (SEQ ID NO:48); the deimmunized sequences are depicted as J415DIVK1 (amino acid residues 18 to 124 of SEQ ID NO:57), J415DIVK2 (SEQ ID NO:62), J415DIVK3 (SEQ ID NO:63), J415DIVK4 (SEQ ID NO:64), J415DIVK5 (SEQ ID NO:50), J415DIVK6 (SEQ ID NO:65), J415DIVK7 (SEQ ID NO:66), and J415DIVK8 (SEQ ID NO:67). The preferred sequence is J415DIVK5 (SEQ ID NO:50). The amino acid replacements are indicated by the boxed residues. A consensus sequence is labeled "majority" (SEQ ID NO:68).

[0095] *Figure 7A* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the deimmunized J415 heavy chain variable region (J415DIVH1) (SEQ ID NO:53-55, respectively). The relative location of the signal sequence, intron and J415DIVH1 amino acid sequence is indicated, as well as some restriction sites.

[0096] *Figure 7B* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the murine J415 heavy chain variable region (SEQ ID NO:125, 47, and 126, respectively). The relative locations of the CDRs and some restriction sites are indicated.

[0097] *Figure 7C* depicts an alignment of the amino acid sequence of the murine J415 heavy chain variable region (SEQ ID NO:47) and a consensus sequence for Kabat subgroup murine VHIIIC (MUVHIII, SEQ ID NO:69). A consensus majority sequence based on the alignment is also shown (SEQ ID NO:70).

[0098] *Figure 8A* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the deimmunized J415 light chain variable region (J415DIVK1) (SEQ ID NO:56-58, respectively). The relative location of the signal sequence, intron and J415DIVK1 amino acid sequence is indicated, as well as some restriction sites.

[0099] *Figure 8B* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the murine J415 light chain variable region (SEQ ID NOs:127, 48, and 128, respectively). The relative locations of the CDRs and some restriction sites are also indicated.

[00100] *Figure 8C* depicts an alignment of the amino acid sequence of the murine J415 light chain variable region (SEQ ID NO:48) and a consensus sequence for Kabat subgroup murine variable light chain (MuVKI, SEQ ID NO:71). A consensus majority sequence based on the alignment is also shown (SEQ ID NO:72).

[00101] *Figure 9A* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the murine J533 heavy chain variable region (SEQ ID NO:73-75, respectively). The relative locations of the CDRs and restriction sites are indicated.

[00102] *Figure 9B* depicts an alignment of the amino acid sequence of the murine J533 heavy chain variable region (SEQ ID NO:74) and a consensus sequence for Kabat subgroup murine variable heavy chain (MuVHIIA, SEQ ID NO:79). A consensus majority sequence based upon the alignment is also shown (SEQ ID NO:80).

[00103] *Figure 10A* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the murine J533 light chain variable region (SEQ ID NO:76-78, respectively). The relative locations of the CDRs and some restriction sites are indicated.

[00104] *Figure 10B* depicts an alignment of the amino acid sequence of the murine J533 light chain variable region (SEQ ID NO:77) and a consensus sequence for Kabat subgroup murine MuVKIII, SEQ ID NO:81). A consensus majority sequence based upon the alignment is also shown (SEQ ID NO:82).

[00105] *Figure 11A* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the murine E99 heavy chain variable

region (SEQ ID NO:83-85, respectively). The relative locations of the CDRs and some restriction sites are indicated.

[00106] *Figure 11B* depicts an alignment of the amino acid sequence of the murine E99 heavy chain variable region (SEQ ID NO:84) and a consensus sequence for Kabat subgroup murine variable heavy chain (MuVHIB, SEQ ID NO:89). A consensus majority sequence based upon the alignment is also shown (SEQ ID NO:90).

[00107] *Figure 12A* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the murine E99 light chain variable region (SEQ ID NO:86-88, respectively). The relative locations of the CDRs and some restriction sites are indicated.

[00108] *Figure 12B* depicts an alignment of the amino acid sequence of the murine E99 light chain variable region (SEQ ID NO:87) and a consensus sequence for Kabat subgroup murine variable light chain (MuVKI, SEQ ID NO:91). A consensus majority sequence based upon the alignment is also shown (SEQ ID NO:92).

[00109] *Figures 13A and B* depict serum PSA levels as a function of time for two patients that were treated with a single dose of ^{90}Y-DOTA-deJ591. Day 0 corresponds to the day on which the ^{90}Y-DOTA-deJ591 was administered.

[00110] *Figure 14* depicts the serum PSA levels as a function of time for a patient that was treated with a single dose of ^{177}Lu-DOTA-deJ591. Day 0 corresponds to the day on which the ^{177}Lu-DOTA-deJ591 was administered.

[00111] *Figure 15* depicts the chemical structures of DM1 and maytansine, a related molecule that lacks the thiol reactive group of DM1 used to conjugate DM1 to antibodies.

DETAILED DESCRIPTION OF THE INVENTION

[00112] This invention provides, *inter alia*, antibodies, e.g., modified antibodies, or antigen-binding fragments thereof, to the extracellular domain of human prostate specific membrane antigen (PSMA). The modified anti-PSMA antibodies, or antigen-binding fragments thereof, have been rendered less immunogenic compared to their unmodified counterparts to a given species, e.g., a human. Human PSMA is expressed on the surface of normal, benign hyperplastic epithelial cells (e.g., benign prostate secretory-acinar epithelium), and cancerous prostate epithelial cells (e.g., prostatic intraepithelial neoplasia and prostatic adenocarcinoma), as

well as vascular endothelial cells proximate to cancerous cells, e.g., renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), pancreatic (e.g., pancreatic duct), melanoma (e.g., malignant melanoma), or soft tissue sarcoma cancerous cells. The antibodies, e.g., the modified antibodies, of the invention bind to the cell surface of cells that express PSMA. PSMA is normally recycled from the cell membrane into the cell. Thus, the antibodies of the invention are internalized with PSMA through the process of PSMA recirculation, thereby permitting delivery of an agent conjugated to the antibody, e.g., a labeling agent, a cytotoxic agent, or a viral particle (e.g., a viral particle containing genes that encode cytotoxic agents, e.g., apoptosis-promoting factors).

[00113] In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

[00114] As used herein, "PSMA" or "prostate-specific membrane antigen" protein refers to mammalian PSMA, preferably human PSMA protein. Human PSMA includes the two protein products, PSMA and PSM', encoded by the two alternatively spliced mRNA variants (containing about 2,653 and 2,387 nucleotides, respectively) of the PSMA cDNA disclosed in Israeli *et al.* (1993) *Cancer Res.* 53:227-230; Su *et al.* (1995) *Cancer Res.* 55:1441-1443; US 5,538,866, US 5,935,818, and WO 97/35616, the contents of which are hereby incorporated by reference. The long transcript of PSMA encodes a protein product of about 100-120 kDa molecular weight characterized as a type II transmembrane receptor having sequence homology with the transferrin receptor and having NAALADase activity (Carter et al. (1996) *Proc. Natl. Acad. Sci.* USA 93:749-753). Accordingly, the term "human PSMA" refers to at least two protein products, human PSMA and PSM', which have or are homologous to (e.g., at least about 85%, 90%, 95% identical to) an amino acid sequence as shown in Israeli *et al.* (1993) *Cancer Res.* 53:227-230; Su *et al.* (1995) *Cancer Res.* 55:1441-1443; US 5,538,866, US 5,935,818, and WO 97/35616; or which is encoded by (a) a naturally occurring human PSMA nucleic acid sequence (e.g., Israeli *et al.* (1993) *Cancer Res.* 53:227-230 or US 5,538,866); (b) a nucleic acid sequence degenerate to a naturally occurring human PSMA sequence; (c) a nucleic acid sequence homologous to (e.g., at least about 85%, 90%, 95% identical to) the naturally occurring human PSMA nucleic acid sequence; or (d) a nucleic acid sequence that hybridizes to one of the foregoing nucleic acid sequences under stringent conditions, e.g., highly stringent conditions.

[00115] An "anti-PSMA antibody" is an antibody that interacts with (e.g., binds to) PSMA, preferably human PSMA protein. Preferably, the anti-PSMA antibody interacts with, e.g., binds to, the extracellular domain of PSMA, e.g., the extracellular domain of human PSMA located at about amino acids 44-750 of human PSMA (amino acid residues correspond to the human PSMA sequence disclosed in US 5,538,866). In one embodiment, the anti-PSMA antibody binds all or part of the epitope of an antibody described herein, e.g., J591, E99, J415, and J533. The anti-PSMA antibody can inhibit, e.g., competitively inhibit, the binding of an antibody described herein, e.g., J591, E99, J415, and J533, to human PSMA. An anti-PSMA antibody may bind to an epitope, e.g., a conformational or a linear epitope, which epitope when bound prevents binding of an antibody described herein, J591, E99, J415, and J533. The epitope can be in close proximity spatially or functionally-associated, e.g., an overlapping or adjacent epitope in linear sequence or conformationally to the one recognized by the J591, E99, J415, or J533 antibody. In one embodiment, the anti-PSMA antibody binds to an epitope located wholly or partially within the region of about amino acids 120 to 500, preferably 130 to 450, more preferably, 134 to 437, or 153 to 347, of human PSMA (amino acid residues correspond to the human PSMA sequence disclosed in US 5,538,866). Preferably, the epitope includes at least one glycosylation site, e.g., at least one N-linked glycosylation site (e.g., an asparagine residue located at about amino acids 190-200, preferably at about amino acid 195, of human PSMA; amino acid residues correspond to the human PSMA sequence disclosed in US 5,538,866).

[00116] Cell lines that produce anti-PSMA antibodies, e.g., murine and modified anti-PSMA antibodies, described herein have been deposited with the ATCC. The ATCC designations of the cell lines that produce each of the anti-PSMA antibodies are listed in Table 7.

Table 7

Anti-PSMA Antibody	ATCC Designation
E99	HB-12101
J415	HB-12109
J533	HB-12127
J591	HB-12126
deJ591	PTA-3709
deJ415	PTA-4174

[00117] In a preferred embodiment, the interaction, e.g., binding, occurs with high affinity (e.g., affinity constant of at least 10^7 M^{-1}, preferably, between 10^8 M^{-1} and 10^{10}, or about 10^9 M^{-1}) and specificity. Preferably, the anti-PSMA antibody treats, e.g., ablates or kills, a cell, e.g., a PSMA-expressing cell (e.g., a prostatic or cancerous cell). The mechanism by which the anti-PSMA antibody treats, e.g., ablates or kills, the cell is not critical to the practice of the invention. In one embodiment, the anti-PSMA antibody may bind to and be internalized with the PSMA expressed in the cells and/or vascular endothelial cells proximate to the cells. In those embodiments, the anti-PSMA antibody can be used to target a second moiety, e.g., a labeling agent, a labeling agent, or a viral agent, to the cell. In other embodiments, the anti-PSMA antibody may mediate host-mediated-killing, e.g., complement- or ADCC-mediated killing, of the cell and/or the vascular cell proximate thereto, upon binding to the extracellular domain of PSMA. The cell can be killed directly by the anti-PSMA antibody by binding directly to the cell or the vascular endothelial cells proximate thereto. Alternatively, the anti-PSMA antibody can treat, e.g., kill or ablate, or otherwise change the properties of the vascular endothelial cells to which it binds so that blood flow to the cells proximate thereto is reduced, thereby causing the cells to be killed or ablated. Examples of anti-PSMA antibodies include, e.g., monospecific, monoclonal (e.g., human), recombinant or modified, e.g., chimeric, CDR-grafted, humanized, deimmunized, and *in vitro* generated anti-PSMA antibodies.

[00118] As used herein, the term "treat" or "treatment" is defined as the application or administration of an anti-PSMA antibody or antigen binding fragment thereof to a subject, e.g., a patient, or application or administration to an isolated tissue or cell from a subject, e.g., a patient, which is returned to the patient. The anti-PSMA antibody or antigen binding fragment thereof, can be administered alone or in combination with, a second agent. The subject can be a patient having a disorder (e.g., a disorder as described herein), a symptom of a disorder or a predisposition toward a disorder. The treatment can be to cure, heal, alleviate, relieve, alter, remedy, ameliorate, palliate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder. While not wishing to be bound by theory treating is believed to cause the inhibition, ablation, or killing of a cell *in vitro* or *in vivo,* or otherwise reducing capacity of a cell, e.g., an aberrant cell, to mediate a disorder, e.g., a disorder as described herein (e.g., a cancer or prostatic disorder).

[00119] As used herein, an amount of an anti-PSMA antibody effective to treat a disorder, or a "therapeutically effective amount" refers to an amount of the antibody which is effective, upon single or multiple dose administration to a subject, in treating a cell, e.g., a prostatic or cancer cell (e.g., a PSMA-expressing prostatic or cancer cell, or a vascular cell proximate thereto), or in prolonging curing, alleviating, relieving or improving a subject with a disorder as described herein beyond that expected in the absence of such treatment. As used herein, "inhibiting the growth" of the neoplasm refers to slowing, interrupting, arresting or stopping its growth and metastases and does not necessarily indicate a total elimination of the neoplastic growth.

[00120] As used herein, an amount of an anti-PSMA antibody effective to prevent a disorder, or a "a prophylactically effective amount" of the antibody refers to an amount of an anti-PSMA antibody, e.g., an anti-PSMA antibody as described herein, which is effective, upon single- or multiple-dose administration to the subject, in preventing or delaying the occurrence of the onset or recurrence of a disorder, e.g., a cancer or prostatic disorder as described herein, or treating a symptom thereof.

[00121] The terms "induce", "inhibit", "potentiate", "elevate", "increase", "decrease" or the like, e.g., which denote quantitative differences between two states, refer to a difference, e.g., a statistically or clinically significant difference, between the two states. For example, "an amount effective to inhibit the proliferation of the PSMA-expressing hyperproliferative cells" means that the rate of growth of the cells will be different, e.g., statistically different, from the untreated cells.

[00122] As used herein, "specific binding" refers to the property of the antibody to: (1) to bind to PSMA, e.g., human PSMA protein, with an affinity of at least $1 \times 10^7\ M^{-1}$, and (2) preferentially bind to PSMA, e.g., human PSMA protein, with an affinity that is at least two-fold, 50-fold, 100-fold, 1000-fold, or more greater than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than PSMA.

[00123] As used herein, the term "antibody" refers to a protein comprising at least one, and preferably two, heavy (H) chain variable regions (abbreviated herein as VH), and at least one and preferably two light (L) chain variable regions (abbreviated herein as VL). The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, termed

"framework regions" (FR). The extent of the framework region and CDRs has been precisely defined (see, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917, which are incorporated herein by reference). Preferably, each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[00124] The VH or VL chain of the antibody can further include all or part of a heavy or light chain constant region. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are inter-connected by, e.g., disulfide bonds. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (Clq) of the classical complement system. The term "antibody" includes intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof), wherein the light chains of the immunoglobulin may be of types kappa or lambda.

[00125] As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 Kd or 214 amino acids) are encoded by a variable region gene at the NH2-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH--terminus. Full-length immunoglobulin "heavy chains" (about 50 Kd or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids). The term "immunoglobulin" includes an immunoglobulin having: CDRs from a non-human source, e.g., from a non-human antibody, e.g., from a mouse immunoglobulin or another non-human immunoglobulin, from a consensus

sequence, or from a sequence generated by phage display, or any other method of generating diversity; and having a framework that is less antigenic in a human than a non-human framework, e.g., in the case of CDRs from a non-human immunoglobulin, less antigenic than the non-human framework from which the non-human CDRs were taken. The framework of the immunoglobulin can be human, humanized non-human, e.g., a mouse, framework modified to decrease antigenicity in humans, or a synthetic framework, e.g., a consensus sequence. These are sometimes referred to herein as modified immunoglobulins. A modified antibody, or antigen binding fragment thereof, includes at least one, two, three or four modified immunoglobulin chains, e.g., at least one or two modified immunoglobulin light and/or at least one or two modified heavy chains. In one embodiment, the modified antibody is a tetramer of two modified heavy immunoglobulin chains and two modified light immunoglobulin chains.

[00126] As used herein, "isotype" refers to the antibody class (e.g., IgM or IgGl) that is encoded by heavy chain constant region genes.

[00127] The term "antigen-binding fragment" of an antibody (or simply "antibody portion," or "fragment"), as used herein, refers to a portion of an antibody which specifically binds to PSMA (e.g., human PSMA), e.g., a molecule in which one or more immunoglobulin chains is not full length but which specifically binds to PSMA (e.g., human PSMA protein). Examples of binding fragments encompassed within the term "antigen-binding fragment" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR) having sufficient framework to specifically bind, e.g., an antigen binding portion of a variable region. An antigen binding portion of a light chain variable region and an antigen binding portion of a hevy chain variable region, e.g., the two domains of the Fv fragment, VL and VH, can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be

encompassed within the term "antigen-binding fragment" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[00128] The term "monospecific antibody" refers to an antibody that displays a single binding specificity and affinity for a particular target, e.g., epitope. This term includes a "monoclonal antibody" or "monoclonal antibody composition," which as used herein refer to a preparation of antibodies or fragments thereof of single molecular composition.

[00129] The term "recombinant" antibody, as used herein, refers to antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial antibody library, antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant antibodies include humanized, CDR grafted, chimeric, deimmunized, *in vitro* generated (e.g., by phage display) antibodies, and may optionally include constant regions derived from human germline immunoglobulin sequences.

[00130] As used herein, the term "substantially identical" (or "substantially homologous") is used herein to refer to a first amino acid or nucleotide sequence that contains a sufficient number of identical or equivalent (e.g., with a similar side chain, e.g., conserved amino acid substitutions) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have similar activities. In the case of antibodies, the second antibody has the same specificity and has at least 50% of the affinity of the same.

[00131] Calculations of "homology" between two sequences cab be performed as follows. The sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide

positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[00132] The comparison of sequences and determination of percent homology between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent homology between two amino acid sequences is determined using the Needleman and Wunsch (1970), *J. Mol. Biol.* 48:444-453 , algorithm which has been incorporated into the GAP program in the GCG software package , using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent homology between two nucleotide sequences is determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a homology limitation of the invention) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

[00133] As used herein, the term "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6, which is incorporated by reference. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency conditions); 2) medium stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; 3) high stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more

washes in 0.2X SSC, 0.1% SDS at 65°C; and preferably 4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very high stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified.

[00134] It is understood that the antibodies and antigen binding fragment thereof of the invention may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on the polypeptide functions. Whether or not a particular substitution will be tolerated, i.e., will not adversely affect desired biological properties, such as binding activity can be determined as described in Bowie, JU et al. (1990) *Science* 247:1306-1310. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[00135] A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of the binding agent, e.g., the antibody, without abolishing or more preferably, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a change.

Anti-PSMA Antibodies

[00136] Many types of anti-PSMA antibodies, or antigen-binding fragments thereof, are useful in the methods of this invention. The antibodies can be of the various isotypes, including: IgG (e.g., IgG1, IgG2, IgG3, IgG4), IgM, IgA1, IgA2, IgD, or IgE. Preferably, the antibody is an IgG isotype, e.g., IgG1. The antibody molecules can be full-length (*e.g.*, an IgG1 or IgG4 antibody) or can include only an antigen-binding fragment (*e.g.*, a Fab, $F(ab')_2$, Fv or a single chain Fv fragment). These include monoclonal antibodies, recombinant antibodies, chimeric antibodies, humanized antibodies, deimmunized antibodies, and human antibodies, as well as antigen-binding fragments of the foregoing.

[00137] Monoclonal anti-PSMA antibodies can be used in the methods of the invention. Preferably, the monoclonal antibodies bind to the extracellular domain of PSMA (i.e., an epitope of PSMA located outside of a cell). Examples of preferred murine monoclonal antibodies to human PSMA include, but are not limited to, E99, J415, J533 and J591, which are produced by hybridoma cell lines having an ATCC Accession Number HB-12101, HB-12109, HB-12127, and HB-12126, respectively, all of which are disclosed in US 6,107,090 and US 6,136,311, the contents of which are expressly incorporated by reference. Most preferably, the murine monoclonal antibody is J591, produced by HB-12126.

[00138] Additional monoclonal antibodies to PSMA can be generated using techniques known in the art. Monoclonal antibodies can be produced by a variety of techniques, including conventional monoclonal antibody methodology e.g., the standard somatic cell hybridization technique of Kohler and Milstein, *Nature* 256: 495 (1975). See generally, Harlow, E. and Lane, D. (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

[00139] Useful immunogens for the purpose of this invention include PSMA (e.g., human PSMA)-bearing cells (e.g., a prostate tumor cell line, e.g., LNCap cells, or fresh or frozen prostate tumor cells); membrane fractions of PSMA-expressing cells (e.g., a prostate tumor cell line, e.g., LNCap cells, or fresh or frozen prostate tumor cells); isolated or purified PSMA, e.g., human PSMA protein (e.g., biochemically isolated PSMA, or a portion thereof, e.g., the extracellular domain of PSMA). Techniques for generating antibodies to PSMA are described in US 6,107,090, US 6,136,311, the contents of all of which are expressly incorporated by reference.

[00140] Human monoclonal antibodies (mAbs) directed against human proteins can be generated using transgenic mice carrying the human immunoglobulin genes rather than the mouse system. Splenocytes from these transgenic mice immunized with the antigen of interest are used to produce hybridomas that secrete human mAbs with specific affinities for epitopes from a human protein (see, e.g., Wood et al. International Application WO 91/00906, Kucherlapati et al. PCT publication WO 91/10741; Lonberg et al. International Application WO 92/03918; Kay et al. International Application 92/03917; Lonberg, N. et al. 1994 *Nature* 368:856-859; Green, L.L. et al. 1994 *Nature Genet.* 7:13-21; Morrison, S.L. et al. 1994 *Proc.*

Natl. Acad. Sci. USA 81:6851-6855; Bruggeman et al. 1993 *Year Immunol* 7:33-40; Tuaillon et al. 1993 *PNAS* 90:3720-3724; Bruggeman et al. 1991 *Eur J Immunol* 21:1323-1326).

[00141] Anti-PSMA antibodies or fragments thereof useful in the present invention may also be recombinant antibodies produced by host cells transformed with DNA encoding immunoglobulin light and heavy chains of a desired antibody. Recombinant antibodies may be produced by known genetic engineering techniques. For example, recombinant antibodies may be produced by cloning a nucleotide sequence, e.g., a cDNA or genomic DNA, encoding the immunoglobulin light and heavy chains of the desired antibody. The nucleotide sequence encoding those polypeptides is then inserted into expression vectors so that both genes are operatively linked to their own transcriptional and translational expression control sequences. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. Typically, both genes are inserted into the same expression vector. Prokaryotic or eukaryotic host cells may be used.

[00142] Expression in eukaryotic host cells is preferred because such cells are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. However, any antibody produced that is inactive due to improper folding may be renaturable according to well known methods (Kim and Baldwin, "Specific Intermediates in the Folding Reactions of Small Proteins and the Mechanism of Protein Folding", *Ann. Rev. Biochem.* 51, pp. 459-89 (1982)). It is possible that the host cells will produce portions of intact antibodies, such as light chain dimers or heavy chain dimers, which also are antibody homologs according to the present invention.

[00143] It will be understood that variations on the above procedure are useful in the present invention. For example, it may be desired to transform a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an antibody. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for PSMA binding, e.g., the constant region may be modified by, for example, deleting specific amino acids. The molecules expressed from such truncated DNA molecules are useful in the methods of this invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are anti-PSMA antibody and the other heavy and light chain are specific for an antigen other than PSMA, or another epitope of PSMA.

[00144] Chimeric antibodies can be produced by recombinant DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted (see Robinson et al., International Patent Publication PCT/US86/02269; Akira, et al., European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., International Application WO 86/01533; Cabilly et al. U.S. Patent No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better et al. (1988 *Science* 240:1041-1043); Liu et al. (1987) *PNAS* 84:3439-3443; Liu et al., 1987, *J. Immunol.* 139:3521-3526; Sun et al. (1987) *PNAS* 84:214-218; Nishimura et al., 1987, *Canc. Res.* 47:999-1005; Wood et al. (1985) *Nature* 314:446-449; and Shaw et al., 1988, *J. Natl Cancer Inst.* 80:1553-1559).

[00145] An antibody or an immunoglobulin chain can be humanized by methods known in the art. Once the murine antibodies are obtained, the variable regions can be sequenced. The location of the CDRs and framework residues can be determined (see, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917, which are incorporated herein by reference). The light and heavy chain variable regions can, optionally, be ligated to corresponding constant regions.

[00146] Murine anti-PSMA antibodies can be sequenced using art-recognized techniques. As an example, hybridomas expressing murine hybridomas J533, J415 and E99 were propagated in culture in RPMI 1640 medium supplemented with 10% fetal calf serum. The isotype of the antibodies secreted was confirmed as IgG1κ, IgG1κ, and IgG3κ respectively. These monoclonal antibodies, like J591, bind to the external domain of prostate specific membrane antigen. J591, J533 and E99 recognize the same epitope, while J415 recognizes an independent epitope. Total RNA for each monoclonal was prepared from 10^7 hybridoma cells. V_H and V_K cDNA was prepared using reverse transcriptase and mouse κ constant region and mouse IgG constant region primers. The first strand cDNAs were amplified by PCR using a variety of mouse signal sequence primers (6 for V_H and 7 for V_K). The amplified DNAs were gel-purified and cloned into the vector pT7Blue. The V_H and V_K clones obtained were screened for correct inserts by

PCR and the DNA sequence of selected clones determined by the dideoxy chain termination method (see Table 7).

[00147] The DNA and amino acid sequences for the heavy and light chain variable regions from J415 were obtained and are shown in Figures 7B (V_H) and 8B (V_K) (also, see Table 5). The location of the CDRs is shown. J415 V_H can be assigned to Mouse Heavy Chains Subgroup IIIC (Kabat EA *et al*; *ibid*). The sequence of J415 V_H compared to the consensus sequence for this subgroup is shown in Figure 7C. J415 V_K can be assigned to Mouse Kappa Chains Subgroup I (Kabat EA *et al*; *ibid*). The sequence of J415 V_K compared to the consensus sequence for this subgroup is shown in Figure 8C.

[00148] The DNA and amino acid sequences encoding the heavy and light chain variable regions J533 were obtained and are shown in Figures 9A (V_H) and 10A (V_K) (see also Table 5). The location of the CDRs is shown in each figure. J533 V_H can be assigned to Mouse Heavy Chains Subgroup IIA (Kabat EA *et al*; Sequences of proteins of Immunological Interest, US Department of Health and Human Services, 1991). The sequence of J533 V_H compared to the consensus sequence for this subgroup is shown in Figure 9B. J533 V_K can be assigned to Mouse Kappa Chains Subgroup III (Kabat EA *et al*; *ibid*). The sequence of J533 V_K compared to the consensus sequence for this subgroup is shown in Figure 10B.

[00149] The DNA and amino acid sequences of the heavy and light chain variable regions of E99 were obtained and are shown in Figures 11A (V_H) and 12A (V_K) (see also Table 5). The location of the CDRs is shown. E99 V_H can be assigned to Mouse Heavy Chains Subgroup IB (Kabat EA *et al*; *ibid*). The sequence of E99 V_H compared to the consensus sequence for this subgroup is shown in Figure 11B. E99 V_K can be assigned Mouse Kappa Chains Subgroup I (Kabat EA *et al*; *ibid*). The sequence of E99 V_K compared to the consensus sequence for this subgroup is shown in Figure 12B.

[00150] The amino acid sequence and nucleotide sequences encoding the variable region of antibodies J415, deJ415, J591, deJ591, J533 and E99 are provided below in Table 8.

Table 8: Antibody variable chain sequences

NAME	Organism	FIG.	SEQ ID NO:	SEQUENCE
V_H J415	Mus musculus	Fig. 7B	125	gaagtgaagcttgaggagtctggaggaggcttggtgcaacctgg aggatccatgaaactctcctgtgttgcctctggattcactttcagtaat

				tactggatgaactgggtccgccagtctccagagaaggggcttgag tgggttgctgaaattagatcgcaatctaataattttgcaacacattatg cggagtctgtgaaagggagggtcatcatctcaagagatgattcca agagtagtgtctacctgcaaatgaacaacttgagagctgaagaca ctggcatttattactgtaccaggcgatggaataatttctggggccaa ggcaccactctcacagtctcctca
V_H Variable Region **J591**	Mus musculus	Fig. 1A	19	EVQLQQSGPELKKPGTSVRISCKTSGYTFT EYTIHWVKQSHGKSLEWIGNINPNNGGTT YNQKFEDKATLTVDKSSSTAYMELRSLTS EDSAVYYCAAGWNFDYWGQGTTLTVSS
V_H **J415** (complementary strand of SEQ ID NO:125)	Mus musculus	Fig. 7B	126	tgaggagactgtgagagtggtgccttggccccagaaattattccat cgcctggtacagtaataaatgccagtgtcttcagctctcaagttgttc atttgcaggtagacactactcttggaatcatctcttgagatgatgacc ctccctttcacagactccgcataatgtgttgcaaaattattagattgc gatctaatttcagcaacccactcaagccccttctctggagactggc ggacccagttcatccagtaattactgaaagtgaatccagaggcaa cacaggagagtttcatggatcctccaggttgcaccaagcctcctcc agactcctcaagcttcacttc
V_L **J415**	Mus musculus	Fig. 8B	127	aacattgtaatgacccaatttcccaaatccatgtccatttcagtagga gagagggtcaccttgacctgcaaggccagtgagaatgtgggtact tatgtgtcctggtatcaacagaaaccagaacagtctcctaagatgtt gatatacggggcatccaaccggttcactggggtccccgatcgctt cacaggcagtggatctgcaacagatttcattctgaccatcagcagt gtgcagactgaagaccttgtagattattactgtggacagagttacac ctttccgtacacgttcggaggggggaccaagctggaaatgaag
V_L Variable Region **J591**	Mus musculus	Fig. 1B	20	DIVMTQSHKFMSTSVGDRVSIICKASQDV GTAVDWYQQKPGQSPKLLIYWASTRHTG VPDRFTGSGSGTDFTLTITNVQSEDLADYF CQQYNSYPLTFGAGTMLDLK
V_L **J415** (complementary strand of SEQ ID NO:127)	Mus musculus	Fig. 8B	128	cttcatttccagcttggtccccccctccgaacgtgtacggaaaggtgt aactctgtccacagtaataatctacaaggtcttcagtctgcacactg ctgatggtcagaatgaaatctgttgcagatccactgcctgtgaagc gatcggggacccccagtgaaccggttggatgccccgtatatcaaca tcttaggagactgttctggtttctgttgataccaggacacataagtac ccacattctcactggccttgcaggtcaaggtgaccctctctcctact gaaatggacatggatttgggaaattgggtcattacaatgtt
V_H Variable Region (Deimm) **J591**	Artificial - deimmunized heavy chain J591	Fig. 2A	21	EVQLVQSGPEVKKPGATVKISCKTSGYTFT EYTIHWVKQAPGKGLEWIGNINPNNGGTT YNQKFEDKATLTVDKSTDTAYMELSSLRS EDTAVYYCAAGWNFDYWGQGTLLTVSS
V_L Variable Region (Deimm) **J591**	Artificial - deimmunized light chain J591	Fig. 2B	22	DIQMTQSPSSLSTSVGDRVTLTCKASQDV GTAVDWYQQKPGPSPKLLIYWASTRHTGI PSRFSGSGSGTDFTLTISSLQPEDFADYYCQ QYNSYPLTFGPGTKVDIK
V_H Deimmunized **J591** CDS (122-166)	Artificial - deimmunized heavy chain J591	Fig. 4A	23	aagcttatgaatatgcaaatcctctgaatctacatggtaaatataggt ttgtctataccacaaacagaaaaacatgagatcacagttctctctac agttactgagcacacaggacctcaccatgggatggagctgtatcat cctcttcttggtagcaacagctacaggtaaggggctcacagtagca ggcttgaggtctggacatatatatgggtgacaatgacatccactttg cctttctctccacaggtgtccactccgaggtccaactggtacagtct

& CDS (249-605)				ggacctgaagtgaagaagcctggggctacagtgaagatatcctg caagacttctggatacacattcactgaatataccatacactgggtga agcaggcccctggaaagggccttgagtggattggaaacatcaatc ctaacaatggtggtaccacctacaatcagaagttcgaggacaagg ccacactaactgtagacaagtccaccgatacagcctacatggagc tcagcagcctaagatctgaggatactgcagtctattattgtgcagct ggttggaactttgactactggggccaagggaccctgctcaccgtct cctcaggtgagtccttacaacctctctcttctattcagcttaaatagat tttactgcatttgttgggggggaaatgtgtgtatctgaatttcaggtca tgaaggactagggacaccttgggagtcagaaagggtcattggga gcccgggctgatgcagacagacatcctcagctcccagacttcatg gccagagatttataggatcc
V_H Deimmunized (complimentary strand of SEQ ID NO:23) **J591**	Artificial - deimmunized heavy chain J591	Fig. 4A	24	ggatcctataaatctctggccatgaagtctgggagctgaggatgtc tgtctgcatcagcccgggctcccaatgaccctttctgactcccaag gtgtccctagtccttcatgacctgaaattcagatacacacatttcccc cccaacaaatgcagtaaaatctatttaagctgaatagaagagagag gttgtaaggactcacctgaggagacggtgagcagggtcccttggc cccagtagtcaaagttccaaccagctgcacaataatagactgcagt atcctcagatcttaggctgctgagctccatgtaggctgtatcggtgg acttgtctacagttagtgtggccttgtcctcgaacttctgattgtaggt ggtaccaccattgttaggattgatgtttccaatccactcaaggcccctt tccaggggcctgcttcacccagtgtatggtatattcagtgaatgtgt atccagaagtcttgcaggatatcttcactgtagccccaggcttcttc acttcaggtccagactgtaccagttggacctcggagtggacacct gtggagagaaaggcaaagtggatgtcattgtcacccatatatatgt ccagacctcaagcctgctactgtgagccccttacctgtagctgttgc taccaagaagaggatgatacagctccatcccatggtgaggtcctgt gtgctcagtaactgtagagagaactgtgatctcatgtttttctgtttgt ggtatagacaaacctatatttaccatgtagattcagaggatttgcata ttcataagctt
V_L Deimmunized **J591** CDS (122-166) & CDS (249-581)	Artificial - deimmunized light chain J591	Fig. 4B	25	aagcttatgaatatgcaaatcctctgaatctacatggtaaatataggt ttgtctataccacaaacagaaaaacatgagatcacagttctctctac agttactgagcacacaggacctcaccatgggatggagctgtatcat cctcttcttggtagcaacagctacaggtaaggggctcacagtagca ggcttgaggtctggacatatatatgggtgacaatgacatccactttg cctttctctccacaggtgtccactccgacatccagatgacccagtct ccctcatccctgtccacatcagtaggagacagggtcaccctcacct gtaaggccagtcaagatgtgggtactgctgtagactggtatcaaca gaaaccaggaccatctcctaaactactgatttattgggcatccactc ggcacactggaatccctagtcgcttctcaggcagtggatctggga cagacttcactctcaccatttctagtcttcagcctgaagactttgcag attattactgtcagcaatataacagctatcctctcacgttcggtcctg gaccaaggtggacatcaaacgtgagtagaatttaaactttgcttc ctcagttggatcc
V_L Deimmunized (complimentary strand of SEQ ID NO:25) **J591**	Artificial - deimmunized light chain J591	Fig. 4B	26	ggatccaactgaggaagcaaagtttaaattctactcacgtttgatgt ccaccttggtcccaggaccgaacgtgagaggatagctgttatattg ctgacagtaataatctgcaaagtcttcaggctgaagactagaaatg gtgagagtgaagtctgtcccagatccactgcctgagaagcgacta gggattccagtgtgccgagtggatgcccaataaatcagtagtttag gagatggtcctggtttctgttgataccagtctacagcagtacccaca tcttgactggccttacaggtgagggtgaccctgtctcctactgatgt

				ggacagggatgagggagactgggtcatctggatgtcggagtgga cacctgtggagagaaaggcaaagtggatgtcattgtcacccatata tatgtccagacctcaagcctgctactgtgagccccttacctgtagct gttgctaccaagaagaggatgatacagctccatcccatggtgagg tcctgtgtgctcagtaactgtagagagaactgtgatctcatgttttct gtttgtggtatagacaaacctatatttaccatgtagattcagaggattt gcatattcataagctt
V_H Deimmunized (predicted a.a. of SEQ ID NO:23) **J591**	Artificial - deimmunized heavy chain J591	Fig. 4A	27	MGWSCIILFLVATATGVHSEVQLVQSGPE VKKPGATVKISCKTSGYTFTEYTIHWVKQ APGKGLEWIGNINPNNGGTTYNQKFEDKA TLTVDKSTDTAYMELSSLRSEDTAVYYCA AGWNFDYWGQGTLLTVSS
V_L Deimmunized (predicted a.a. of SEQ ID NO:25) **J591**	Artificial - deimmunized light chain J591	Fig. 4B	28	MGWSCIILFLVATATGVHSDIQMTQSPSSL STSVGDRVTLTCKASQDVGTAVDWYQQK PGPSPKLLIYCASTRHTGIPSRFSGSGSGTD FTLTISSLQPEDFADYYCQQYNSYPLTFGP GTKVDIK
V_H Variable Region **J415**	Mus musculus	Fig. 5	47	EVKLEESGGGLVQPGGSMKLSCVASGFTF SNYWMNWVRQSPEKGLEWVAEIRSQSNN FATHYAESVKGRVIISRDDSKSSVYLQMN NLRAEDTGIYYCTRRWNNFWGQGTTLTV SS
V_L Variable Region **J415**	Mus musculus	Fig. 6	48	NIVMTQFPKSMSISVGERVTLTCKASENVG TYVSWYQQKPEQSPKMLIYGASNRFTGVP DRFTGSGSATDFILTISSVQTEDLVDYYCG QSYTFPYTFGGGTKLEMK
V_H Variable Region (Deimm) **J415-4**	Artificial - deimmunized heavy chain J415-4	Fig. 5	49	EVKLEESGGGLVQPGGSMKISCVASGFTFS NYWMNWVRQSPEKGLEWVAEIRSQSNNF ATHYAESVKGRVIISRDDSKSSVYLQMNS LRAEDTAVYYCTRRWNNFWGQGTTVTVS S
V_L Variable Region (Deimm) **J415-5**	Artificial - deimmunized light chain J415-5	Fig. 6	50	NIVMTQFPKSMSASAGERMTLTCKASENV GTYVSWYQQKPTQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTISSVQAEDLVDYYC GQSYTFPYTFGGGTKLEMK
V_H Deimmunized **J415-4**	Artificial - deimmunized heavy chain J415-4		51	gaagtgaaacttgaggagtctggaggaggcttggtgcaacctgg agggtccatgaaaatctcctgtgttgcctctggattcactttcagtaa ttactggatgaactgggtccgccagtctccagagaaggggcttga gtgggttgctgaaattagatcgcaatctaataattttgcaacacattat gcggagtctgtgaaagggagggtcatcatctcaagagatgattcc aagagtagtgtctacctgcaaatgaacagtttgagagctgaagaca ctgccgtttattactgtaccaggcgatggaataatttctggggccaa ggcaccactgtcacagtctcctca
V_L Deimmunized	Artificial - deimmunized		52	aacattgtaatgacccaatttcccaaatccatgtccgcctcagcagg agagaggatgaccttgacctgcaaggccagtgagaatgtgggta

J415-5	light chain J415-5			cttatgtgtcctggtatcaacagaaaccaacacagtctcctaagatg ttgatatacggggcatccaaccggttcactggggtcccagatcgct tctccggcagtggatctggaacagatttcattctgaccatcagcagt gtgcaggcagaagaccttgtagattattactgtggacagagttaca cctttccgtacacgttcggaggggggaccaagctggaaatgaag
V_H Deimmunized **J415-1** CDS (122-160) & CDS (249-608) Mature (18-133)	Artificial - deimmunized heavy chain J415-1	Fig. 7A	53	aagcttatgaatatgcaaatcctctgaatctacatggtaaatataggt ttgtctataccacaaacagaaaaacatgagatcacagttctctctac agttactgagcacacaggacctcaccatgggatggagctgtatca tcctcttcttggtagcaacagctacaggtaaggggctcacagtagc aggcttgaggtctggacatatatatgggtgacaatgacatccactt gcctttctctccacaggtgtccactccgaagtgaaacttgaggagt ctggaggaggcttggtgcaacctggagggtccatgaaaatctcct gtaaagcctctggattcactttcagtaattactggatgaactgggtcc gccagactccagagaaggggcttgagtgggttgctcttattagatc gcaatctaataattttgcaacacattatgcggagtctgtgaaaggga gggtcatcatctcaagagatgattccaagagtagtgtctacctgca aatgaacagtttgagagctgaagacactgccgtttattactgtacca ggcgatggaataatttctggggccaaggcaccactgtcacagtct cctcaggtgagtccttacaacctctctcttctattcagcttaaatagat tttactgcatttgttgggggggaaatgtgtgtatctgaatttcaggtca tgaaggactagggacaccttgggagtcagaaagggtcattggga gcccgggctgatgcagacagacatcctcagctcccagacttcatg gccagagatttataggatcc
V_H Deimmunized (predicted a.a. of SEQ ID NO:53) **J415-1**	Artificial - deimmunized heavy chain J415-1	Fig. 7A	54	MGWSCIILFLVATGVHSEVKLEESGGGLV QPGGSMKISCKASGFTFSNYWMNWVRQT PEKGLEWVALIRSQSNNFATHYAESVKGR VIISRDDSKSSVYLQMNSLRAEDTAVYYC TRRWNNFWGQGTTVTVSS
V_H Deimmunized (complimentary strand of SEQ ID NO:53) **J415-1**	Artificial - deimmunized heavy chain J415-1	Fig. 7A	55	ggatcctataaatctctggccatgaagtctgggagctgaggatgtc tgtctgcatcagcccgggctcccaatgacccttttctgactcccaag gtgtccctagtccttcatgacctgaaattcagatacacacatttcccc cccaacaaatgcagtaaaatctatttaagctgaatagaagagagag gttgtaaggactcacctgaggagactgtgacagtggtgccttggc cccagaaattattccatcgcctggtacagtaataaacggcagtgtct tcagctctcaaactgttcatttgcaggtagacactactcttggaatca tctcttgagatgatgaccctccctttcacagactccgcataatgtgtt gcaaaattattagattgcgatctaataagagcaacccactcaagcc ccttctctggagtctggcggacccagttcatccagtaattactgaaa gtgaatccagaggctttacaggagattttcatggaccctccaggttg caccaagcctcctccagactcctcaagtttcacttcggagtggaca cctgtggagagaaaggcaaagtggatgtcattgtcacccatatata tgtccagacctcaagcctgctactgtgagccccttacctgtagctgt tgctaccaagaagaggatgatacagctccatcccatggtgaggtc ctgtgtgctcagtaactgtagagagaactgtgatctcatgtttttctgt ttgtggtatagacaaacctatatttaccatgtagattcagaggatttg catattcataagctt
V_L Deimmunized	Artificial - deimmunized	Fig. 8A	56	aagcttatgaatatgcaaatcctctgaatctacatggtaaatataggt ttgtctataccacaaacagaaaaacatgagatcacagttctctctac

J415-1 CDS (122-160) & CDS (249-581)	light chain J415-1			agttactgagcacacaggacctcaccatgggatggagctgtatca tcctcttcttggtagcaacagctacaggtaaggggctcacagtagc aggcttgaggtctggacatatatatgggtgacaatgacatccacttt gcctttctctccacaggtgtccactccaacattgtaatgacccaatc ccccaaatccatgtccgcctcagcaggagagaggatgaccttgac ctgcaaggccagtgagaattccggtacttatgtgtcctggtatcaac agaaaccaacacagtctcctaagatgttgatatacggggcatccaa ccggttcactggggtcccagatcgcttctccggcagtggatctgga acagatttcattctgaccgccagcagtgtgcaggcagaagaccct gtagattattactgtggacagagttacacctttccgtacacgttcgga gggggaccaagctggaaatgaagcgtgagtagaatttaaacttt gcttcctcagttggatcc
V_L Deimmunized (predicted a.a. of SEQ ID NO:56) **J415-1**	Artificial - deimmunized light chain J415-1	Fig. 8A	57	MGWSCIILFLVATGVHSNIVMTQSPKSMS ASAGERMTLTCKASENSGTYVSWYQQKP TQSPKMLIYGASNRFTGVPDRFSGSGSGTD FILTASSVQAEDPVDYYCGQSYTFPYTFGG GTKLEMK
V_L Deimmunized (complimentary strand of SEQ ID NO:56) **J415-1**	Artificial - deimmunized light chain J415-1	Fig. 8A	58	ggatccaactgaggaagcaaagtttaaattctactcacgcttcatttc cagcttggtccccctccgaacgtgtacggaaaggtgtaactctgt ccacagtaataatctacagggtcttctgcctgcacactgctggcgg tcagaatgaaatctgttccagatccactgccggagaagcgatctgg gaccccagtgaaccggttggatgccccgtatatcaacatcttagga gactgtgttggtttctgttgataccaggacacataagtaccggaatt ctcactggccttgcaggtcaaggtcatcctctctcctgctgaggcg gacatggatttgggggattgggtcattacaatgttggagtggacac ctgtggagagaaaggcaaagtggatgtcattgtcacccatatatat gtccagacctcaagcctgctactgtgagccccttacctgtagctgtt gctaccaagaagaggatgatacagctccatcccatggtgaggtcc tgtgtgctcagtaactgtagagagaactgtgatctcatgtttttctgttt gtggtatagacaaacctatatttaccatgtagattcagaggatttgca tattcataagctt
V_H Deimmunized **J415-2**	Artificial - deimmunized heavy chain J415-2	Fig. 5	59	EVKLEESGGGLVQPGGSMKISCVASGFTFS NYWMNWVRQTPEKGLEWVALIRSQSNNF ATHYAESVKGRVIISRDDSKSSVYLQMNS LRAEDTAVYYCTRRWNNFWGQGTTVTVS S
V_H Deimmunized **J415-3**	Artificial - deimmunized heavy chain J415-3	Fig. 5	60	EVKLEESGGGLVQPGGSMKISCVASGFTFS NYWMNWVRQTPEKGLEWVAEIRSQSNNF ATHYAESVKGRVIISRDDSKSSVYLQMNS LRAEDTAVYYCTRRWNNFWGQGTTVTVS S
J415 V_H (DI) majority sequence	Artificial - majority sequence	Fig. 5	61	EVKLEESGGGLVQPGGSMKISCVASGFTFS NYWMNWVRQTPEKGLEWVAEIRSQSNNF ATHYAESVKGRVIISRDDSKSSVYLQMNS LRAEDTAVYYCTRRWNNFWGQGTTVTVS S
V_L Deimmunized **J415-2**	Artificial - deimmunized light chain	Fig. 6	62	NIVMTQSPKSMSASAGERMTLTCKASENV GTYVSWYQQKPTQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTASSVQAEDPVDYYC

	J415-2			GQSYTFPYTFGGGTKLEMK
V_L Deimmunized **J415-3**	Artificial - deimmunized light chain J415-3	Fig. 6	63	NIVMTQSPKSMSASAGERMTLTCKASENV GTYVSWYQQKPTQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTASSVQAEDLVDYYC GQSYTFPYTFGGGTKLEMK
V_L Deimmunized **J415-4**	Artificial - deimmunized light chain J415-4	Fig. 6	64	NIVMTQSPKSMSASAGERMTLTCKASENV GTYVSWYQQKPTQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTISSVQAEDLVDYYC GQSYTFPYTFGGGTKLEMK
V_L Deimmunized **J415-6**	Artificial - deimmunized light chain J415-6	Fig. 6	65	NIVMTQFPKSMSASAGERMTLTCKASENV GTYVSWYQQKPEQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTISSVQAEDLVDYYC GQSYTFPYTFGGGTKLEMK
V_L Deimmunized **J415-7**	Artificial - deimmunized light chain J415-7	Fig. 6	66	NIVMTQFPKSMSASAGERVTLTCKASENV GTYVSWYQQKPTQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTISSVQAEDLVDYYC GQSYTFPYTFGGGTKLEMK
V_L Deimmunized **J415-8**	Artificial - deimmunized light chain J415-8	Fig. 6	67	NIVMTQFPKSMSASAGERMTLTCKASENS GTYVSWYQQKPEQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTISSVQAEDLVDYYC GQSYTFPYTFGGGTKLEMK
J415 V_L (DI) *majority* sequence	Artificial - *majority* sequence	Fig. 6	68	NIVMTQFPKSMSASAGERMTLTCKASENV GTYVSWYQQKPTQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTISSVQAEDLVDYYC GQSYTFPYTFGGGTKLEMK
MuV_HIIIC	Mus musculus	Fig. 7C	69	EVKLEESGGGLVQPGGSMKLSCVASGFTF SNYWMNWVRQSPEKGLEWVAEIRLKSDN YATHYAESVKGRFTISRDDSKSSVYLQMN NLRAEDTGIYYCTTGGYGGRRSWFAYWG QGTLVTVSS
J415V_H/MuV_HIIIC majority sequence	Artificial - majority sequence	Fig. 7C	70	EVKLEESGGGLVQPGGSMKLSCVASGFTF SNYWMNWVRQSPEKGLEWVAEIRLQSDN FATHYAESVKGRVIISRDDSKSSVYLQMN NLRAEDTGIYYCTTGGYGGRRSWNAFWG QGTLVTVSS
MuV_L1	Mus musculus	Fig. 8C	71	DIVMTQSPSSLAVSAGEKVTMSCKSSQSLL NSGNQKNYLAWYQQKPGQSPKLLIYWAS TRESGVPDRFTGSGSGTDFTLTISSVQAED LAVYYCQNDYSYPLTFGAGTKLELK
J415V_L/MuV_L1 majority sequence	Artificial - majority sequence	Fig. 8C	72	DIVMTQSPSSLAVSAGEKVTLSCKASESLL NVGNQKTYVAWYQQKPGQSPKLLIYGAS TRESGVPDRFTGSGSGTDFILTISSVQAEDL AVYYCGNSYSFPLTFGGGTKLELK
J533 V_H CDS (1-354)	Mus musculus	Fig. 9A	73	gaggtccagctgcagcagtctggacctgagctggttaagcctggg gcttcagtgaagatgtcctgcaaggcttctggatacacattcactgg ctatgttatgcactgggtgaagcagaagcctggacaggtccttgag tggattggatatattaatccttacaatgatgttactaggtataatggga agttcaaaggcaaggccacactgacctcagacaaatattccagca cagcctacatggagctcagcggcctgacctctgaggactctgcgg tctattactgtgcaagaggggagaactggtactactttgactcctgg

				ggccgaggcgccactctcacagtctcctca
J533 V_H (predicted amino acid of SEQ ID NO:73)	Mus musculus	Fig. 9A	74	EVQLQQSGPELVKPGASVKMSCKASGYTF TGYVMHWVKQKPGQVLEWIGYINPYNDV TRYNGKFKGKATLTSDKYSSTAYMELSGL TSEDSAVYYCARGENWYYFDSWGRGATL TVSS
J533 V_H (complementary strand of SEQ ID NO:73)	Mus musculus	Fig. 9A	75	tgaggagactgtgagagtggcgcctcggccccaggagtcaaagt agtaccagttctcccctcttgcacagtaatagaccgcagagtcctc agaggtcaggccgctgagctccatgtaggctgtgctggaatatttg tctgaggtcagtgtggccttgcctttgaacttcccattatacctagta acatcattgtaaggattaatatatccaatccactcaaggacctgtcc aggcttctgcttcacccagtgcataacatagccagtgaatgtgtatc cagaagccttgcaggacatcttcactgaagccccaggcttaacca gctcaggtccagactgctgcagctggacctc
J533 V_L CDS (1-333)	Mus musculus	Fig. 10A	76	gacattgtgctgacccaatctccagcttctttggctgtgtctctagga cagagggccaccatatcctgcagagccagtgaaagtattgatagtt atgacaatacttttatgcactggtaccagcagaaaccaggacagcc acccaacctcctcatctttcgtgcatccatcctagaatctgggatcc ctgccaggttcagtggcagtgggtctgggacagacttcaccctca ccatttatcctgtggaggctgatgatgttgcaacctattactgtcacc aaagtattgaggatccgtacacgttcggaggggggaccaagctg gaaataaaa
J533 V_L (predicted amino acid of SEQ ID NO:76)	Mus musculus	Fig. 10A	77	DIVLTQSPASLAVSLGQRATISCRASESIDS YDNTFMHWYQQKPGQPPNLLIFRASILES GIPARFSGSGSGTDFTLTIYPVEADDVATY YCHQSIEDPYTFGGGTKLEIK
J533 V_L (complementary strand of SEQ ID NO:76)	Mus musculus	Fig. 10A	78	ttttatttccagcttggtccccccctccgaacgtgtacggatcctcaat actttggtgacagtaataggttgcaacatcatcagcctccacaggat aaatggtgagggtgaagtctgtcccagacccactgccactgaacc tggcagggatcccagattctaggatggatgcacgaaagatgagg aggttgggtggctgtcctggtttctgctggtaccagtgcataaaagt attgtcataactatcaatactttcactggctctgcaggatatggtggc cctctgtcctagagacacagccaaagaagctggagattgggtcag cacaatgtc
MuV_HII	Mus musculus	Fig. 9B	79	EVQLQQSGPELVKPGASVKISCKASGYTFT DYYMNNWVKQSPGKSLEWIGDINPGNGG TSYNQKFKGKATLTVDKSSSTAYMQLSSL TSEDSAVYYCARGYYSSSYMAYYAFDYW GQGTTVTVSS
$J533V_H/MuV_HII$ majority sequence	Artificial - majority sequence	Fig. 9B	80	EVQLQQSGPELVKPGASVKISCKASGYTFT GYVMNNWVKQSPGQVLEWIGDINPGNGG TSYNGKFKGKATLTVDKSSSTAYMELSGL TSEDSAVYYCARGENSSSYMAYYAFDSW GQGATVTVSS
MuV_L-3	Mus musculus	Fig. 10B	81	DIVLTQSPASLAVSLGQRATISCRASESVDS YGNSFMHWYQQKPGQPPKLLIYAASNLES GVPARFSGSGSGTDFTLNIHPVEEDDAATY YCQQSNEDPPWTFGGGTKLEIK
$J533V_L/$	Artificial -	Fig.	82	DIVLTQSPASLAVSLGQRATISCRASESVDS

MuV_L-3 majority sequence	*majority* sequence	10B		YGNSFMHWYQQKPGQPPNLLIFAASILES GVPARFSGSGSGTDFTLTIHPVEADDAATY YCQQSIEDPPYTFGGGTKLEIK
E99 V_H CDS (1-363)	Mus musculus	Fig. 11A	83	caggtgcagctaaaggagtcaggacctggcctggtggcgtcctc acagagcctgtccatcacatgcaccgtctcaggattctcattaacc gcctatggtattaactgggttcgccagcctccaggaaagggtctgg agtggctgggagtgatatggcctgatggaaacacagactataattc aactctcaaatccagactgaacatcttcaaggacaactccaagaac caagttttcttaaaaatgagcagtttccaaactgatgacacagccag atacttctgtgccagagattcgtatggtaacttcaagagggttggtt tgacttctggggccagggcaccactctcacagtctcctca
E99 V_H (predicted amino acid of SEQ ID NO:83)	Mus musculus	Fig. 11A	84	QVQLKESGPGLVASSQSLSITCTVSGFSLT AYGINWVRQPPGKGLEWLGVIWPDGNTD YNSTLKSRLNIFKDNSKNQVFLKMSSFQT DDTARYFCARDSYGNFKRGWFDFWGQGT TLTVSS
E99 V_H (complementary strand of SEQ ID NO:83)	Mus musculus	Fig. 11A	85	tgaggagactgtgagagtggtgccctggccccagaagtcaaacc aaccctcttgaagttaccatacgaatctctggcacagaagtatctg gctgtgtcatcagtttggaaactgctcatttttaagaaaacttggttctt ggagttgtccttgaagatgttcagtctggatttgagagttgaattata gtctgtgtttccatcaggccatatcactcccagccactccagaccct ttcctggaggctggcgaacccagttaataccataggcggttaatga gaatcctgagacggtgcatgtgatggacaggctctgtgaggacgc caccaggccaggtcctgactcctttagctgcacctg
E99 V_L CDS (1-321)	Mus musculus	Fig. 12A	86	aacattgtgatgacccagtctcaaaaattcatgtccacatcaccagg agacagggtcagggtcacctgcaaggccagtcagaatgtgggtt ctgatgtagcctggtatcaagcgaaaccaggacaatctcctagaat actgatttactcgacatcctaccgttacagtggggtccctgatcgctt cacagcctatggatctgggacagatttcactctcaccattaccaatg tgcagtctgaagacttgacagagtatttctgtcagcaatataatagct atcctctcacgttcggtgctgggaccaagctggagctgaaa
E99 V_L (predicted amino acid of SEQ ID NO:86)	Mus musculus	Fig. 12A	87	NIVMTQSQKFMSTSPGDRVRVTCKASQNV GSDVAWYQAKPGQSPRILIYSTSYRYSGVP DRFTAYGSGTDFTLTITNVQSEDLTEYFCQ QYNSYPLTFGAGTKLELK
E99 V_L (complementary strand of SEQ ID NO:86)	Mus musculus	Fig. 12A	88	tttcagctccagcttggtcccagcaccgaacgtgagaggatagcta ttatattgctgacagaaatactctgtcaagtcttcagactgcacattg gtaatggtgagagtgaaatctgtcccagatccataggctgtgaagc gatcagggaccccactgtaacggtaggatgtcgagtaaatcagta ttctaggagattgtcctggtttcgcttgataccaggctacatcagaac ccacattctgactggccttgcaggtgaccctgaccctgtctcctggt gatgtggacatgaatttttgagactgggtcatcacaatgtt
MuV_HIB	Mus musculus	Fig. 11B	89	QVQLKESGPGLVASSQSLSITCTVSGFSLT AYGINWVRQPPGKGLEWLGVIWPDGNTD YNSTLKSRLNIFKDNSKNQVFLKMSSFQT DDTARYFCARDSYGNFKRGWFDFWGQGT TLTVSS
$E99V_H/MuV_H$ IB	Artificial - majority	Fig. 11B	90	QVQLKESGPGLVASSQSLSITCTVSGFSLT AYGINWVRQPPGKGLEWLGVIWPDGNTD

majority sequence	sequence			YNSTLKSRLNIFKDNSKNQVFLKMSSFQT DDTARYFCARDSYGNFKRGWFDFWGQGT TLTVSS
MuV_L-1	Mus musculus	Fig. 12B	91	DIVMTQSPSSLAVSAGEKVTMSCKSSQSLL NSGNQKNYLAWYQQKPGQSPKLLIYWAS TRESGVPDRFTGSGSGTDFTLTISSVQAED LAVYYCQNDYSYPLTFGAGTKLELK
$E99V_L/MuV_L$ -1 majority sequence	Artificial - majority sequence	Fig. 12B	92	DIVMTQSQSSLAVSAGDKVTVSCKASQSL LNVGSDKNYVAWYQAKPGQSPKLLIYSAS TRESGVPDRFTGSGSGTDFTLTISSVQAED LAVYFCQNDNSYPLTFGAGTKLELKRA

[00151] Humanized or CDR-grafted antibody molecules or immunoglobulins can be produced by CDR-grafting or CDR substitution, wherein one, two, or all CDRs of an immunoglobulin chain can be replaced. See e.g., U.S. Patent 5,225,539; Jones et al. 1986 *Nature* 321:552-525; Verhoeyan et al. 1988 *Science* 239:1534; Beidler et al. 1988 *J. Immunol.* 141:4053-4060; Winter US 5,225,539, the contents of all of which are hereby expressly incorporated by reference.

[00152] Winter describes a CDR-grafting method that may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987; Winter US 5,225,539), the contents of which is expressly incorporated by reference. All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to a predetermined antigen.

[00153] Humanized antibodies can be generated by replacing sequences of the Fv variable region that are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General methods for generating humanized antibodies are provided by Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi et al., 1986, *BioTechniques* 4:214, and by Queen et al. US 5,585,089, US 5,693,761 and US 5,693,762, the contents of all of which are hereby incorporated by reference. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, may be obtained from a hybridoma producing an antibody against a predetermined target, as described above. The recombinant DNA encoding

the humanized antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

[00154] Also within the scope of the invention are humanized antibodies in which specific amino acids have been substituted, deleted or added. In particular, preferred humanized antibodies have amino acid substitutions in the framework region, such as to improve binding to the antigen. For example, a selected, small number of acceptor framework residues of the humanized immunoglobulin chain can be replaced by the corresponding donor amino acids. Preferred locations of the substitutions include amino acid residues adjacent to the CDR, or which are capable of interacting with a CDR (see e.g., US 5,585,089). Criteria for selecting amino acids from the donor are described in US 5,585,089, e.g., columns 12-16 of US 5,585,089, the contents of which are hereby incorporated by reference. The acceptor framework can be a mature human antibody framework sequence or a consensus sequence.

[00155] As used herein, the term "consensus sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related sequences (See e.g., Winnaker, From Genes to Clones (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of proteins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence. A "consensus framework" refers to the framework region in the consensus immunoglobulin sequence.

[00156] Other techniques for humanizing antibodies are described in Padlan et al. EP 519596 A1, published on December 23, 1992.

[00157] The anti-PSMA antibody, or antigen fragment thereof, may also be modified by specific deletion of human T cell epitopes or "deimmunization" by the methods disclosed in WO 98/52976 and WO 00/34317, the contents of which are specifically incorporated by reference herein. Briefly, the murine heavy and light chain variable regions of an anti-PSMA antibody can be analyzed for peptides that bind to MHC Class II; these peptides represent potential T-cell epitopes (as defined in WO 98/52976 and WO 00/34317). For detection of potential T-cell epitopes, a computer modelling approach termed "peptide threading" can be applied, and in addition a database of human MHC class II binding peptides can be searched for motifs present in the murine V_H and V_L sequences, as described in WO 98/52976 and WO 00/34317. These motifs bind to any of the 18 major MHC class II DR allotypes, and thus constitute potential T

cell epitopes. Potential T-cell epitopes detected can be eliminated by substituting small numbers of amino acid residues in the variable regions, or preferably, by single amino acid substitutions. As far as possible conservative substitutions are made, often but not exclusively, an amino acid common at this position in human germline antibody sequences may be used. Human germline sequences are disclosed in TomliNS0n, I.A. *et al.* (1992) *J. Mol. Biol.* 227:776-798; Cook, G. P. *et al.* (1995) *Immunol. Today* Vol. 16 (5): 237-242; Chothia, D. *et al.* (1992) *J. Mol. Bio.* 227:799-817. The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by TomliNS0n, I.A. et al. MRC Centre for Protein Engineering, Cambridge, UK). After the deimmunized V_H and V_L of an anti-PSMA antibody are constructed by mutagenesis of the murine V_H and V_L genes. The mutagenized variable sequence can, optionally, be fused to a human constant region, e.g., human IgG1 or κ constant regions.

[00158] In some cases a potential T cell epitope will include residues which are known or predicted to be important for antibody function. For example, potential T cell epitopes are usually biased towards the CDRs. In addition, potential T cell epitopes can occur in framework residues important for antibody structure and binding. Changes to eliminate these potential epitopes will in some cases require more scrutiny, e.g., by making and testing chains with and without the change. Where possible, potential T cell epitopes that overlap the CDRs were eliminated by substitutions outside the CDRs. In some cases, an alteration within a CDR is the only option, and thus variants with and without this substitution should be tested. In other cases, the substitution required to remove a potential T cell epitope is at a residue position within the framework that might be critical for antibody binding. In these cases, variants with and without this substitution should be tested. Thus, in some cases several variant deimmunized heavy and light chain variable regions were designed and various heavy/light chain combinations tested in order to identify the optimal deimmunized antibody. The choice of the final deimmunized antibody can then be made by considering the binding affinity of the different variants in conjunction with the extent of deimmunization, i.e., the number of potential T cell epitopes remaining in the variable region.

[00159] The recombinant deimmunized antibody can be transfected into a suitable host cell for expression, for example, NS0 or CHO cells, to produce complete recombinant antibodies.

[00160] In one embodiment, deimmunized V_H and V_L of murine J591 regions were constructed by mutagenesis of the murine V_H and V_L genes. The murine J591 variable region sequences are shown in Figures 1A-1B. Potential epitopes (identified using a peptide threading program) in murine J591 heavy chain and light chain variable regions are shown in Figures 3A and 3B, respectively. The 13-mer peptides predicted to bind to MHC class II are indicated by the underline, the CDRs are located at residues 26 to 35, 50 to 66, and 99 to 104 of Figure 3A and residues 24 to 34, 50 to 56, and 89 to 97 of Figure 3B, and residues altered in the deimmunized heavy and light chain variable regions are boxed. Where possible, amino acid substitutions are those commonly used in human germline heavy and light chain variable regions. In addition to the *in silico* analysis using the peptide threading software, a database of human MHC class II binding peptides was searched for motifs present in the murine J591 sequence.

[00161] The following 13-mers (labelled by first linear residue number of the 13-mer) of the murine J591 heavy chain variable region were predicted to bind to MHC Class II were 2, 10, 16, 30, 32, 35, 43, 46, 58, 62, 70, 81, 84, 91, and 100 (Figure 3A). An explanation of the rationale behind changes made to the residues in the murine J591 heavy chain variable region is set forth below (note residues altered are identified under the Kabat numbering system):

5Q→V removes the potential epitope at residue 2;

11,12LV→VK remove the potential epitope at residue 10;

12V→K is also changed to remove a motif from the database of human MHC class II binding peptides;

16,17TS→AT, and 19R→K remove the potential epitope at residue 16;

the epitope at residue 30 spans CDR1 and is therefore unaltered;

40,41SH→AP removes potential epitopes at residues 32 and 35;

44S→G reduces binding score for epitope at 43, this 13 mer spans CDR2;

the epitopes at residues 46, 58 and 62 span CDR2, and are thus unaltered;

75,76SS→TD remove the potential epitope at residue 70;

82aR→S, 83T→R remove potential epitopes at residues 81and 84;

87S→T this change made to remove a motif from the database of human MHC class II binding peptides;

the epitope at residue 91 spans CDR3 and is therefore unaltered; and

108T→L removes the potential epitope at residue 100.

[00162] The following 13-mers (labelled by first linear residue number of the 13-mer) of the murine J591 light chain variable region that were predicted to bind to MHC Class II molecules were 1, 8, 17, 27, 30, 31, 35, 45, 47, 56, 60, 71, 73, 81, 94 (Figure 3B). An explanation of the rationale behind changes made to the residues in the murine J591 light chain variable region is set forth below (note residues altered are identified under the Kabat numbering system):

3V→Q removes potential epitope at residue 1;

8-11HKFM→PSSL removes potential epitope at residue 8(13);

20-22SII→TLT removes potential epitopes at residues 17 and 20;

21I→L is also changed to remove a motif from the database of human MHC class II binding peptides;

the epitope at residue 27 spans CDR1 and is therefore unaltered;

42Q→P reduces the binding score for the epitope at residue 31;

the epitopes at residues 44 and 47 span CDR2 and are thus unaltered;

58V→I is changed to remove a motif from the database of human MHC class II binding peptides;

60D→S, 62T→S removes the epitopes at residues 56 and 60;

76-78TNV→SSL, 80S→P, 83L→F removes the epitopes at residues 71, 73, 76, and 81;

87F→Y I is changed to remove a motif from the database of human MHC class II binding peptides;

100 A→P and 103 M→K remove the epitope at residue 94; and

104 L→V and 106 L→I are changed to remove a motif from the database of human MHC class II binding peptides.

[00163] The amino acid and nucleotide sequences for the deimmunized J591 heavy and light chain variable regions are shown in Figures 2A-2B and 4A-4B, respectively (see also Table 8).

[00164] Human IgG1 or κ constant regions were added and the composite genes transfected into NS0 cells to produce complete recombinant anti-PSMA antibodies. These

antibodies bound to PSMA (on LNCap cells) as efficiently as the original murine antibody, and have reduced or no immunogenicity in man.

[00165] The design of deimmunized J415 was similar to the making of the deimmunized J591 antibody. The heavy and light chain sequences were cloned from the hybridoma designated HB-12109. These sequences were cloned, sequenced and expressed as a chimaeric antibody for use as a control antibody. The murine V region sequences were subjected to peptide threading to identify potential T cell epitopes, through analysis of binding to 18 different human MHC class II allotypes. The results of the peptide threading analysis for the murine sequences are shown in Table 9.

Table 9: Potential T cell epitopes in murine J415 sequences

Sequence	Number of potential T cell	Location of potential epitopes+ (no. of potential MHC binders from 18 groups tested)
Murine J415 V_H	12	10(17), 16(13), 21(9), 30(6), 35(16), 43(8), 46(6), 49(8), 64(6), 80(15), 86(15), 104(6)
Murine J415 V_K	13	5(5), 11(18), 13(11), 17(5), 27(8), 31(7), 56(15), 60(12), 70(5), 71(11), 73(17), 76(7), 81(17)

+first amino acid of potential epitope, numbering E or N amino acid number1 to S or K amino acid number 107 and 116 for V_H and V_K respectively.

[00166] Primary deimmunized V_H and V_L sequences were defined (J415DlVH1, J415DlVKl). As generation of the primary deimmunized sequences requires a small number of amino acid substitutions that might affect the binding of the final deimmunized molecule, three other variant V_HS and seven other V_LS were designed (see Figures 5 and 6). The nucleotide sequences for the primary deimmunized V_H and V_L regions are shown in Figures 7A and 8A, respectively. Comparisons of the amino acid sequences of the murine and deimmunized V regions of J415 are shown in Figure 5 for V_H and Figure 6 for V_L.

[00167] An explanation of the rational behind some of the changes made to the residues in the murine J415 heavy chain variable region is set forth below (note residues altered are identified according to the linear numbering shown in Figure 5):

20L→I removes the potential epitope at residues 10 and 16;

87N→S removes the potential epitopes at residues 80 and 86;

94,95GI→AV remove the potential epitope at residue 86; and

112L→V removes the potential epitope at residue 104.

[00168] An explanation of the rational behind some of the changes made to the residues in the murine J415 light chain variable region is set forth below (note residues altered are identified according to the linear numbering shown in Figure 6):

13I A removes the potential epitopes at residues 5, 11 and 13;

15V A removes the potential epitopes at residues 5, 11, and 13;

19V-M removes the potential epitopes at residues 11, 13, and 17;

41E-T removes the potential epitope at residue 31;

63T-S removes the potential epitopes at residues 56 and 60;

68A-G removes the potential epitopes at residues 56 and 60; and

80T-A removes the potential epitopes at residues 70, 71, 73, and 76;

[00169] The deimmunized variable regions for J415 were constructed by the method of overlapping PCR recombination. The cloned murine V_H and V_K genes were used as templates for mutagenesis of the framework regions to the required deimmunized sequences. Sets of mutagenic primer pairs were synthesized encompassing the regions to be altered. The vectors VH-PCR1 and VK-PCR1 (Riechmann *et al.* (1988) *Nature* 332:323-7) were used as templates to introduce 5' flanking sequence including the leader signal peptide, leader intron and the murine immunoglobulin promoter, and 3' flanking sequence including the splice site, and intron sequences. The deimmunized V regions produced were cloned into pUC19 and the entire DNA sequence was confirmed to be correct for each deimmunized V_H and V_L.

[00170] The deimmunized heavy and light chain V-region genes were excised from pUC19 as *Hind*III to *Bam*HI fragments, which include the murine heavy chain immunoglobulin promoter, the leader signal peptide, leader intron, the V_H or V_L sequence and the splice site. These were transferred to the expression vectors pSV*gpt* and pSV*hyg*, which include human IgG1 or κ constant regions respectively and markers for selection in mammalian cells. The DNA sequence was confirmed to be correct for the deimmunized V_H and V_L in the expression vectors.

[00171] For the transfection of expression vectors pSVgptJ415VHHuIgG1 and pSVhygJ415VKHuCK into NS0 (a non-immunoglobulin producing mouse myeloma, obtained from the European Collection of Animal Cell Cultures, Porton UK (ECACC No. 85110503)) cells, 3 and 6 ug of plasmid DNA respectively was prepared and then linearized with *Pvu*I to

improve transfection efficiency. The ethanol precipitated DNA was then mixed with a semi-confluent flask of NS0 cells that had been harvested by centrifugation and resuspended in 0.5 ml of non-selective Dulbecco's Modified Eagle's Medium (DMEM)(Life Technologies Inc.) in a 0.4 cm gene pulser cuvette. The cells and DNA were chilled on ice for 5 minutes before a pulse of 17OV, 960μF was applied. The cuvette was returned to ice for a further 20 minutes before being transferred to a 75cm^2 flask containing 20mls non-selective DMEM to recover for 24 hours. The cells were then harvested and resuspended in selective DMEM and plated over 4x96 well plates, 200 μl/well. A similar protocol was followed for the transfection of expression vectors encoding the deJ591 antibody heavy chain and light chain subunits into NS0 cells.

[00172] To culture, select, and expand NS0 cell lines, the cells are grown at 37°C, 5%CO_2 and 10% FBS. To prepare non-selective medium for routine culture of NS0 cells, the culture medium is Dulbecco's Modification of Eagle's Medium (DMEM)(Life Technologies, Catalogue No: 31965-023) supplemented with 10% fetal bovine serum of USA origin (Life Technologies, Fetal Bovine Serum Cat No: 16000), Antibiotic/Antimycotic solution (Life Technologies, Cat No: 15240), Gentamycin (Life Technologies, catalogue No: 15710), Sodium pyruvate (Life Technologies, Catalogue No: 11360-039). When growing NS0 cells up to saturation for antibody production do not add the xanthine and mycophenolic acid and the FBS is reduced to 5%.

[00173] To prepare selective medium for culture of NS0 transfectomas, the culture medium is Dulbecco's Modification of Eagle's Medium (DMEM)(Life Technologies, Catalogue No: 31965-023) supplemented with 10% foetal bovine serum of USA origin (Life Technologies, Fetal Bovine Serum Cat No: 16000), Antibiotic/Antimycotic solution (Life Technologies, Cat No: 15240), Gentamycin (Life Technologies, catalogue No: 15710), Sodium pyruvate (Life Technologies, Catalogue No: 11360-039), 250 μg/ml xanthine (Sigma Catalogue No: X-3627, stock made up at 25 mg/ml in 0.5M NaOH), and 0.8 μg/ml mycophenolic acid (Sigma Catalogue No: M-3536, stock made up at 2.5 mg/ml in 100% ethanol).

[00174] After approximately 10 days the cell colonies expressing the *gpt* gene were visible to the naked eye. The plates were then screened for antibody production using the following protocol for human IgGl/κ Screening ELISA. 6 single colonies were picked from wells with high ODs greater than 0.7 into a 24 well cell culture plate. Within 5-6 days the cells were expanded into a 25cm^2 flask. The antibody productivity of the selected clones was assayed using

the following protocol for human IgG1/κ ELISA from saturated cultures in the 24 well and 25cm^2 flasks.

[00175] The details of the protocol are as follows. ELISA plates (Dynatech Immulon 2) are coated with 100 µL per well with sheep α human κ antibody (The Binding Site Cat No: AU015) diluted 1:1000 in carbonate/bicarbonate coating buffer pH9.6 (Sigma Cat: C-3041). The coated plate is incubated at 4°C overnight or 1 hr at 37°C. The plate is then washed 3 times with PBST (PBS with 0.05%Tween 20). The samples are added, 100 µL per well from 24 well plates, 25 µL +75 µL PBST for 96 well plates. Blank wells are treated with PBST only. The reaction mixture is incubated at room temperature for 1 hr. Then, the plate is wash 3 times with PBST (PBS with 0.05%Tween 20). The secondary antibody, peroxidase conjugated sheep α human IgG γ chain specific is added (The Binding Site Cat No: APO04) at a ratio of 1:1000 in PBST, 100 µL per well. The mixture is incubated at room temperature for l hour. The mixture is then washed 3 times with PBST (PBS with 0.05%Tween 20).

[00176] To make up the substrate, one tablet (20 mg)of OPD (o-PHENYLENE DIAMINE) (Sigma Cat No: P-7288) is dissolved in 45 ml of H_20 plus 5ml 10 x peroxidase buffer (make 10 x peroxidase buffer with Sigma phosphate citrate buffer tablets pH 5.0, P-4809), add 10 µL 30%(w/w) hydrogen peroxide (Sigma Cat No: H1109) just before use. The substrate is then added at 100µL per well and incubate RT for 5 min or as required. When the color develops, the reaction can be stopped by adding 25 µL 12.5% H_2SO_4. The results are read at 492 nm.

Expression and expansion of J591 and J415 Deimmunized Antibodies

[00177] The clones with the highest productivity were expanded into a 75 cm^2 flask and then into 2x 175 cm^2 flasks. The cells from one of the 175 cm^2 flask was used to inoculate 4x triple layer flasks (500 cm^2, Nalge Nunc International) containing non selective DMEM containing 5% FBS, cells from the other were frozen as detailed in the protocol for freezing NS0 cells detailed below.

[00178] To cryoprotect mammalian cells and resurrect cells from liquid nitrogen, the following materials are needed: Fetal Bovine serum (Life Technologies Cat No: 16000), DMSO (Sigma, Cat No: D4540), 2 ml cryotubes (Nunc or Greiner), and polystyrene box with walls 1 - 2

cm thick. Briefly, actively growing cells are harvested by centrifugation (1000 rpm, 5 min) and resuspended at about 10^7 cells/ml in 10% DMS0/90% FBS. As a rough guide, cells grown to a semi-confluency should be resuspended in 1 ml for a 75 cm^2 flask or 2.5 ml for a 175 cm^2 flask. A required number of tubes are cooled and labeled in ice. 1 ml portions are aliquoted to labeled cryotubes. The cryotubes are placed in polystyrene box at –70°C for at least 4 h, or overnight. The vials are transferred to canes and place in liquid nitrogen. A record of the storage should be made both in the canister index and the central cell line indexing system.

[00179] To thaw the cells from liquid nitrogen, the vial is removed from liquid nitrogen and contents are thawed quickly by incubation at 37°C, while swirling in a waterbath. The outside of the vial is cleaned with 70% methylated spirits. The suspension is transferred to a universal container. 10 ml of the medium to be used to propagate the cell line is added dropwise, swirling to mix. The cells are harvested by centrifugation (1000 rpm, 5 min). The supernatant is discarded. The cells are resuspended in 20 ml growth medium and transfer to a 75 cm^2 flask. If low viability is suspected, extra serum can be added to the growth medium to 20%, use only 5 ml, and transfer to a 25 cm^2 flask.

[00180] After 10-14 days the 500 ml to 1 liter static saturated cultures were harvested. Antibody was purified, by ProSepA (Millipore Ltd.) affinity chromatography using the following protocol for antibody purification. The purified antibody preparation was sterilized by filtration and stored at 4°C.

[00181] The antibody purification protocol is as follows: NS0 transfectoma cell line producing antibody is grown in DMEM 5% FCS in Nunc Triple layer flasks, 250 ml per flask (total volume 1L) for 10 - 14 days until nearing saturation. Conditioned medium collected and spun at 3000 rpm for 5 min in bench centrifuge 5 mins to remove cells. $1/10^{th}$ volume 1M Tris-HCl pH 8 (Sigma Cat: T3038) is then added to cell supernatant to make this 0.1 M Tris-HCl pH8. 0.5 to 1 ml Prosep A (Millipore Cat: 113 111824) is added and stirred overnight at room temperature. Prosep A collected by spinning at 3000 rpm for 5 mins then packed into a Biorad Poly-Prep column (Cat: 73 1-1550). The column is washed with 10ml PBS, then eluted in 1 ml fractions with 0.1M Glycine pH 3.0. Each fraction is collected into a tube containing 100 microL 1M Tris-HCl pH 8 (Sigma, as above). Absorbance of each fraction is measured at 280 nm. Fractions containing the antibody are pooled and dialyzed against PBS overnight at room temperature. The preparation is sterilized by filtration through a 0.2 micron syringe filter and the

absorbance of each fraction is measured at 280nm. The antibody concentration is determined by ELISA for human IgG.

[00182] The purified antibody can be quantified using the protocol for Human IgGl/κ ELISA described above.

Testing of J415 Deimmunized antibodies

[00183] The J415 deimmunized antibodies (including various combinations of the deimmunized light chain and heavy chain subunits) were tested in an ELISA for binding to LNCap membrane preparation following the protocol as detailed above. ELISA plates were coated with LNCap membrane preparation and blocked with 5% BSA in phosphate buffered saline. Doubling dilutions of the J415 chimeric antibody (murine variable heavy and light chain regions fused to human constant heavy and light chain regions, respectivley) and the deimmunized antibodies were applied. Detection was with horseradish peroxidase conjugated goat anti-human IgG and donkey anti-mouse for chimeric and mouse antibodies respectively. Color was developed with o-phenylene diamine substrate.

[00184] The antibody composed of deimmunized J415 heavy chain version 4 combined with deimmunized J415 light chain version 5 shows equivalent binding to LNCap cells as compared to the chimeric antibody. Also, when DIVK5 is combined with heavy chain versions 1 and 2, binding to LNCap cells is equivalent to that of the chimeric antibody when tissue culture supernantant is analyzed. These data can be confirmed with purified antibody. When light chains 1, 2, 3 were combined with any of the J415 heavy chain versions 1, 2, 3, and 4 no antibody was produced. Deimmunized J415 light chain versions 1, 2, and 3 may be defective on structural grounds. The best chain combination for higher affinity and decreased immunogenicity is DIVH4/DIVK5.

[00185] The antibody composed of deimmunized heavy chain version 4 combined with deimmunized light chain version 5 showed equivalent binding to LNCap compared to the chimeric antibody. Also, when DIVK5 is combined with heavy chain versions 1 and 2, binding to LNCap cells is two-fold less than that of the chimeric when purified antibody is analyzed.

[00186] Monoclonal anti-PSMA antibodies can also be generated by other methods known to those skilled in the art of recombinant DNA technology.

[00187] Anti-PSMA antibodies that are not intact antibodies are also useful in this invention. Such antibodies may be derived from any of the antibodies described above. For example, antigen-binding fragments, as well as full-length monomeric, dimeric or trimeric polypeptides derived from the above-described antibodies are themselves useful. Useful antibody homologs of this type include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR), e.g., one or more isolated CDRs together with sufficient framework to provide an antigen binding fragment. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding fragment" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[00188] Antibody fragments may also be produced by chemical methods, e.g., by cleaving an intact antibody with a protease, such as pepsin or papain, and optionally treating the cleaved product with a reducing agent. Alternatively, useful fragments may be produced by using host cells transformed with truncated heavy and/or light chain genes.

[00189] Monoclonal, chimeric, humanized, deimmunized antibodies, which have been modified by, e.g., deleting, adding, or substituting other portions of the antibody, e.g., the constant region, are also within the scope of the invention. For example, an antibody can be modified as follows: (i) by deleting the constant region; (ii) by replacing the constant region with another constant region, e.g., a constant region meant to increase half-life, stability or affinity of the antibody, or a constant region from another species or antibody class; or (iii) by modifying one or more amino acids in the constant region to alter, for example, the number of

glycosylation sites, effector cell function, Fc receptor (FcR) binding, complement fixation, among others.

[00190] In one embodiment, the constant region of the antibody can be replaced by another constant region from, e.g., a different species. This replacement can be carried out using molecular biology techniques. For example, the nucleic acid encoding the VL or VH region of a antibody can be converted to a full-length light or heavy chain gene, respectively, by operatively linking the VH or VL-encoding nucleic acid to another nucleic acid encoding the light or heavy chain constant regions. The sequences of human light and heavy chain constant region genes are known in the art. Preferably, the constant region is human, but constant species from other species, e.g., rodent (e.g., mouse or rat), primate, camel, rabbit can also be used. Constant regions from these species are known in the art (see *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

[00191] Methods for altering an antibody constant region are known in the art. Antibodies with altered function, e.g. altered affinity for an effector ligand, such as FcR on a cell, or the C1 component of complement can be produced by replacing at least one amino acid residue in the constant portion of the antibody with a different residue (see e.g., EP 388,151 A1, US 5,624,821 and US 5,648,260, the contents of all of which are hereby incorporated by reference). Similar type of alterations could be described which if applied to the murine, or other species immunoglobulin would reduce or eliminate these functions.

[00192] An anti-PSMA antibody, or antigen-binding fragment thereof, can be derivatized or linked to another functional molecule (e.g., another peptide or protein). Accordingly, the antibodies and antibody portions of the invention are intended to include derivatized and otherwise modified forms of the antibodies described herein, including immunoadhesion molecules. For example, an antibody or antibody portion of the invention can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (*e.g.*, a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

[00193] One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, *e.g.*, to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (*e.g.*, m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (*e.g.*, disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, IL.

[00194] Useful detectable agents with which an antibody or antibody portion of the invention may be derivatized (or labeled) to include fluorescent compounds, various enzymes, prosthetic groups, luminescent materials, bioluminescent materials, fluorescent emitting metal atoms, e.g., europium (Eu), and other anthanides, and radioactive materials (described below). Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, β-galactosidase, acetylcholinesterase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody may also be derivatized with a prosthetic group (e.g., streptavidin/biotin and avidin/biotin). For example, an antibody may be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding. Examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of bioluminescent materials include luciferase, luciferin, and aequorin.

[00195] Labeled antibodies can be used, for example, diagnostically and/or experimentally in a number of contexts, including (i) to isolate a predetermined antigen by standard techniques, such as affinity chromatography or immunoprecipitation; (ii) to detect a predetermined antigen (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein; (iii) to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to determine the efficacy of a given treatment regimen.

[00196] An anti-PSMA antibody or antigen-binding fragment thereof may be conjugated to a another molecular entity, typically a label or a therapeutic (e.g., a cytotoxic or cytostatic) agent or moiety.

[00197] Radioactive isotopes can be used in diagnostic or therapeutic applications. Radioactive isotopes that can be coupled to the anti-PSMA antibodies include, but are not limited to α-, β-, or γ-emitters, or β- and γ-emitters. Such radioactive isotopes include, but are not limited to iodine (^{131}I or ^{125}I), yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), praseodymium, astatine (^{211}At), rhenium (^{186}Re), bismuth (^{212}Bi or ^{213}Bi), indium (^{111}In), technetium (^{99}mTc), phosphorus (^{32}P), rhodium (^{188}Rh), sulfur (^{35}S), carbon (^{14}C), tritium (^{3}H), chromium (^{51}Cr), chlorine (^{36}Cl), cobalt (^{57}Co or ^{58}Co), iron (^{59}Fe), selenium (^{75}Se), or gallium (^{67}Ga). Radioisotopes useful as therapeutic agents include yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), praseodymium, astatine (^{211}At), rhenium (^{186}Re), bismuth (^{212}Bi or ^{213}Bi), and rhodium (^{188}Rh). Radioisotopes useful as labels, e.g., for use in diagnostics, include iodine (^{131}I or ^{125}I), indium (^{111}In), technetium (^{99}mTc), phosphorus (^{32}P), carbon (^{14}C), and tritium (^{3}H), or one or more of the therapeutic isotopes listed above.

[00198] The invention provides radiolabeled anti-PSMA antibodies and methods of labeling the same. In one embodiment, a method of labeling an anti-PSMA antibody is disclosed. The method includes contacting an anti-PSMA antibody, e.g. an anti-PSMA antibody described herein, with a chelating agent, e.g., 1,4,7,10-tetraazacyclododecane-N, N', N'',N'''-tetraacetic acid (DOTA), to thereby produced a conjugated antibody. The conjugated antibody is radiolabeled with a radioisotope, e.g., ^{111}Indium, ^{90}Yttrium and ^{177}Lutetium, to thereby produce a labeled anti-PSMA antibody. Detailed procedures for radiolabeling an anti-PSMA antibody are described in more detail in the sections below and the appended examples. For example, the anti-PSMA antibodies can be radiolabeled with ^{111}Indium, ^{90}Yttrium, or ^{177}Lutetium by coupling with 1,4,7,10-tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid (DOTA) as described in USSN 60/295,214, filed on June 1, 2001, the contents of which are incorporated by reference in its entirety. Detailed experimental protocols for chelating anti-PSMA antibodies are described in Example 16 of USSN 60/295,214, which is specifically incorporated by reference in the present application and is reproduced below as Example 1.

[00199] As is discussed above the antibody can be conjugated to a therapeutic agent. Therapeutically active radioisotopes have already been mentioned. Examples of other

therapeutic agents include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, *e.g.*, maytansinol (see US Patent No. 5,208,020), CC-1065 (see US Patent Nos. 5,475,092, 5,585,499, 5,846,545) and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, CC-1065, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine, vinblastine, taxol and maytansinoids).

[00200] The conjugates of the invention can be used for modifying a given biological response. The therapuetic agent is not to be construed as limited to classical chemical therapeutic agents. For example, the therapeutic agent may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, diphtheria toxin, or a component thereof (e.g., a component of pseudomonas exotoxin is PE38); a protein such as tumor necrosis factor, interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors. Similarly, the therapeutic agent can be a viral particle, e.g., a recombinant viral particle, that is conjugated (e.g., via a chemical linker) or fused (e.g., via a viral coat protein) to an anit-PSMA anitbody of the invention. Introduction of the viral nucleic acid molecules, e.g., recombinant viral nucleic acid molecules, into cells, e.g., prostate cancer cells or vascular endothelial cells associated with tumors, that express PSMA can occur following binding and endocytosis of the anti-PSMA antibody/viral particle conjugate or fusion.

Nucleic Acids, Vectors and Host Cells

[00201] Another aspect of the invention pertains to isolated nucleic acid, vector and host cell compositions that can be used for recombinant expression of the modified antibodies and antigen-binding fragment of the invention. In one embodiment, a first and second isolated nucleic acid comprising a nucleotide sequence encoding heavy and light chain variable regions, respectively, of an anti-PSMA antibody, e.g., a modified anti-PSMA antibody (e.g., a deimmunized J591 or J415 anti-PSMA antibody), or an antigen fragment thereof, are provided.

[00202] The nucleotide and amino acid sequence of the modified (deimmunized) anti-PSMA J591 immunoglobulin light chain variable region is shown in Figures 4B (SEQ ID NO:25 and 22, respectively). The non-coding complementary nucleotide sequence is also shown in Figure 4B (SEQ ID NO:26). The J591 deimmunized anti-PSMA antibody light chain variable region contains the following regions: an FR1 domain corresponding to about amino acid residues 1-23 of SEQ ID NO:22 (linear numbering; see also SEQ ID NO:13), which is encoded by about nucleotides 261-329 of SEQ ID NO:25; a CDR1 domain corresponding to about amino acid residues 24-34 of SEQ ID NO:22 (linear numbering; see also SEQ ID NO:4), which is encoded by about nucleotides 330-362 of SEQ ID NO:25; an FR2 domain corresponding to about amino acid residues 35-49 of SEQ ID NO:22 (linear numbering; see also SEQ ID NO:14), which is encoded by about nucleotides 363-407 of SEQ ID NO:25; a CDR2 domain corresponding to about amino acid residues 50-56 of SEQ ID NO:22 (linear numbering; see SEQ ID NO:5), which is encoded by about nucleotides 408-428 of SEQ ID NO:25; an FR3 domain corresponding to about amino acid residues 57-88 of SEQ ID NO:22 (linear numbering; see also SEQ ID NO:15), which is encoded by about nucleotides 429-524 of SEQ ID NO:25; a CDR3 domain corresponding to about amino acid residues 89-97 of SEQ ID NO:22 (linear numbering; see also SEQ ID NO:6), which is encoded by about nucleotides 525-551 of SEQ ID NO:25; and an FR4 domain corresponding to about amino acid residues 98-107 of SEQ ID NO:22 (linear numbering; see also SEQ ID NO:16), which is encoded by about nucleotides 552-581 of SEQ ID NO:25.

[00203] The nucleotide and amino acid sequence of the modified (deimmunized) anti-PSMA J591 immunoglobulin heavy chain variable region is shown in Figure 4A (SEQ ID NO:23 and 21, respectively). The non-coding complementary sequence is also shown in Figure 4A (SEQ ID NO:24). The J591 deimmunized anti-PSMA antibody heavy chain variable region

contains the following regions: an FR1 domain corresponding to about amino acid residues 1-25 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:9), which is encoded by about nucleotides 261-335 of SEQ ID NO:23; a CDR1 domain corresponding to about amino acid residues 26-35 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:1), which is encoded by about nucleotides 336-365 of SEQ ID NO:23; an FR2 domain corresponding to about amino acid residues 36-49 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:10), which is encoded by about nucleotides 366-407 of SEQ ID NO:23; a CDR2 domain of corresponding to about amino acid residues 50-66 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:2), which is encoded by about nucleotides 408-458 of SEQ ID NO:23; an FR3 domain corresponding to about amino acid residues 67-98 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:11), which is encoded by about nucleotides 459-554 of SEQ ID NO:23; a CDR3 domain corresponding to about amino acid residues 99-104 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:3), which is encoded by about nucleotides 555-572 of SEQ ID NO:23; and an FR4 domain corresponding to about amino acid residues 105-115 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:9), which is encoded by about nucleotides 573-605 of SEQ ID NO:23.

[00204] The nucleotide and amino acid sequence of the modified (deimmunized) anti-PSMA J415 immunoglobulin light chain variable region (J415DIVK1) is shown in Figure 8A (SEQ ID NO:56 and 57, respectively). The non-coding complementary nucleotide sequence of J415DIVK1 is also shown in Figure 8A (SEQ ID NO:58). The J415 deimmunized anti-PSMA antibody light chain variable region contains the following regions: an FR1 domain corresponding to about amino acid residues 1-23 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:41), which is encoded by about nucleotides 261-329 of SEQ ID NO:56; a CDR1 domain corresponding to about amino acid residues 24-34 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:32), which is encoded by about nucleotides 330-362 of SEQ ID NO:56; an FR2 domain corresponding to about amino acid residues 35-49 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:42), which is encoded by about nucleotides 363-407 of SEQ ID NO:56; a CDR2 domain corresponding to about amino acid residues 50-56 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:33), which is encoded by about nucleotides 408-428 of SEQ ID NO:56; an FR3 domain corresponding to about amino acid residues 57-88 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:43), which is encoded by about nucleotides 429-

524 of SEQ ID NO:56; a CDR3 domain corresponding to about amino acid residues 89-97 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:34), which is encoded by about nucleotides 525-551 of SEQ ID NO:56; and an FR4 domain corresponding to about amino acid residues 98-107 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:44), which is encoded by about nucleotides 552-581 of SEQ ID NO:56. The nucleotide and amino acid sequences of the preferred modified (deimmunized) anti-PSMA J415 immunoglobulin light chain variable region (J415DIVK5) are shown in SEQ ID NO:50 and 52, respectively; J415DIVK5 can be broken down into its component sequences in a manner identical to that shown above for J415DIVK1.

[00205] The nucleotide and amino acid sequence of the modified (deimmunized) anti-PSMA J415 immunoglobulin heavy chain variable region is shown in Figure 7A (SEQ ID NO:53 and 54, respectively). The non-coding complementary sequence is also shown in Figure 7A (SEQ ID NO:55). The J415 deimmunized anti-PSMA antibody heavy chain variable region contains the following regions: an FR1 domain corresponding to about amino acid residues 1-25 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:37), which is encoded by about nucleotides 261-335 of SEQ ID NO:53; a CDR1 domain corresponding to about amino acid residues 26-35 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:29), which is encoded by about nucleotides 336-365 of SEQ ID NO:53; an FR2 domain corresponding to about amino acid residues 36-49 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:38), which is encoded by about nucleotides 366-407 of SEQ ID NO:53; a CDR2 domain corresponding to about amino acid residues 50-68 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:30), which is encoded by about nucleotides 408-464 of SEQ ID NO:53; an FR3 domain corresponding to about amino acid residues 69-100 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:39), which is encoded by about nucleotides 465-560 of SEQ ID NO:53; a CDR3 domain corresponding to about amino acid residues 101-105 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:31), which is encoded by about nucleotides 561-575 of SEQ ID NO:53; and an FR4 domain corresponding to about amino acid residues 106-116 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:40), which is encoded by about nucleotides 576-608 of SEQ ID NO:53. The nucleotide and amino acid sequences of the preferred modified (deimmunized) anti-PSMA J415 immunoglobulin heavy chain variable region (J415DIVH4) are

shown in SEQ ID NO:51 and 49, respectively; J415DIVH4 can be broken down into its component sequences in a manner identical to that shown above for J415DIVH1.

[00206] It will be appreciated by the skilled artisan that nucleotide sequences encoding anti-PSMA modified antibodies (*e.g.*, FR domains, e.g., FR1-4), can be derived from the nucleotide and amino acid sequences described in the present application using the genetic code and standard molecular biology techniques.

[00207] In one embodiment, the isolated nucleic acid comprises an anti-PSMA modified antibody heavy chain variable region nucleotide sequence having a nucleotide sequence as shown in Figure 4A (SEQ ID NO:23), Figure 7A (SEQ ID NO:53) or SEQ ID NO:51 (for J415DIVH4) or a complement thereof (e.g., SEQ ID NO:24 or SEQ ID NO:55), the nucleotide sequence of the heavy chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174 or a complement thereof; a sequence at least 85%, 90%, 95%, 99% or more identity thereto; or a sequence capable of hybridizing under stringent conditions described herein (e.g., highly stringent conditions) to a nucleotide sequence shown in Figure 4A (SEQ ID NO:23), Figure 7A (SEQ ID NO:53), SEQ ID NO:51, or a complement thereof (e.g., SEQ ID NO:24 or SEQ ID NO:55), or the nucleotide sequence of the heavy chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174, or a complement thereof.

[00208] In another embodiment, the isolated nucleic acid encodes an anti-PSMA modified antibody heavy chain variable region amino acid sequence having an amino acid sequence as shown in Figure 2A (SEQ ID NO:21) or Figure 5 (e.g., SEQ ID NO:49), or the amino acid sequence of the heavy chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174; a sequence at least 85%, 90%, 95%, 99% or more identical thereto; or a sequence capable of hybridizing under stringent conditions described herein (e.g., highly stringent conditions) to a nucleotide sequence encoding the amino acid sequence as shown in Figure 2A (SEQ ID NO:21), Figure 5 (e.g., SEQ ID NO:49), or the amino acid sequence of the heavy chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

[00209] In another embodiment, the isolated nucleic acid comprises a nucleotide sequence encoding at least one, preferably two, and most preferably three, CDRs of the heavy chain variable region of the anti-PSMA antibody chosen from the amino acid sequences of SEQ ID

NO:1, 2, and 3, or 29, 30 and 31, or 93, 94, and 95, or 99, 100 and 101, or a CDR sequence which differs by one or two amino acids from the sequences described herein. In yet another embodiment, the isolated nucleic acid comprises a nucleotide sequence encoding CDRs 1, 2, or 3 shown in Figure 4A (SEQ ID NO:23), in SEQ ID NO:51, in Figure 7B (SEQ ID NO:125), in Figure 9A (SEQ ID NO:73), or in Figure 11A (SEQ ID NO:83), or a complement thereof, or a sequence encoding a CDR that differs by one or two amino acids from the sequences described herein.

[00210] In another embodiment, the isolated nucleic acid comprises a nucleotide sequence encoding at least one, preferably two, three and most preferably four amino acid sequences from the heavy chain variable framework region of the anti-PSMA modified antibody chosen from SEQ ID NO:9, 10, 11 and 12, or 37, 38, 39 and 40, or a sequence at least 85%, 90%, 95%, 99% or more identical thereto.

[00211] In yet another embodiment, the isolated nucleic acid comprises an anti-PSMA modified antibody light chain variable region nucleotide sequence having a sequence as shown in Figure 4B (SEQ ID NO:25), Figure 8A (SEQ ID NO:56), or SEQ ID NO:52, or a complement thereof (e.g., SEQ ID NO:26 or 58), or the nucleotide sequence of the light chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174; a sequence at least 85%, 90%, 95%, 99% or more identical thereto; or a sequence capable of hybridizing under stringent conditions described herein (e.g., highly stringent conditions) to the nucleotide sequence as shown in Figure 4B (SEQ ID NO:25), Figure 8A (SEQ ID NO:56), SEQ ID NO:52, or a complement thereof (e.g., SEQ ID NO:26 or 58), or the nucleotide sequence of the light chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174, or a complement thereof. In another embodiment, the isolated nucleic acid encodes an anti-PSMA modified antibody light chain variable region amino acid sequence having a sequence as shown in Figure 2B (SEQ ID NO:22) or in Figure 6 (e.g., SEQ ID NO:50), the amino acid sequence of the light chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174; a sequence at least 85%, 90%, 95%, 99% or more identity thereto; or a sequence capable of hybridizing under stringent conditions described herein (e.g., highly stringent conditions) to a nucleotide sequence encoding the amino acid sequence as shown in Figure 2B (SEQ ID NO:22) or in Figure 6 (SEQ ID NO:50), or the amino acid sequence of the

light chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

[00212] In another embodiment, the isolated nucleic acid comprises a nucleotide sequence encoding at least one, preferably two, and most preferably three, CDRs of the light chain variable region of the anti-PSMA antibody chosen from the amino acid sequences of SEQ ID NO:4, 5, and 6, or 32, 33, and 34, or 96, 97, and 98, or 102, 103, and 104, or a sequence encoding a CDR which differs by one or two amino acids from the sequences described herein.

[00213] In yet another embodiment, the isolated nucleic acid comprises a nucleotide sequence selected encoding CDRs 1-3 of the light chain variable nucleotide sequence shown in SEQ ID NO:25, or a sequence encoding a CDR which differs by one or two amino acids from the sequences described herein. In another embodiment, the isolated nucleic acid comprises a nucleotide sequence encoding at least one, preferably two, three and most preferably four amino acid sequences from the light chain variable framework region of the anti-PSMA modified antibody chosen from SEQ ID NO:13, 14, 15, and 16, or 41, 42, 43, and 44, or a sequence at least 85%, 90%, 95%, 99% or more identical thereto.

[00214] In a preferred embodiment, there is an isolated first and second nucleic acid which have nucleotide sequences encoding a light chain and the heavy chain variable regions of an anti-PSMA antibody, respectively, wherein each isolated nucleic acid has at least one, two, three, four, five and preferably all CDRs chosen from the amino acid sequences of SEQ ID NO:1, 2, 3, 4, 5, and 6, or 29, 30, 31, 32, 33 and 34, or 93, 94, 95, 96, 97, and 98, or 99, 100, 101, 102, 103, and 104, or sequence encoding a CDR which differs by one or two amino acids from the sequences described herein.

[00215] The nucleic acid can encode only the light chain or the heavy chain variable region, or can also encode an antibody light or heavy chain constant region, operatively linked to the corresponding variable region. In one embodiment, the light chain variable region is linked to a constant region chosen from a kappa or a lambda constant region. Preferably, the light chain constant region is from a lambda type (e.g., a human type lambda). In another embodiment, the heavy chain variable region is linked to a heavy chain constant region of an antibody isotype selected from the group consisting of IgG (e.g., IgG1, IgG2, IgG3, IgG4), IgM, IgA1, IgA2, IgD, and IgE. Preferably, the heavy chain constant region is from an IgG (e.g., an IgG1) isotype, e.g., a human IgG1.

[00216] Nucleic acids of the invention can be chosen for having codons, which are preferred, or non-preferred, for a particular expression system. E.g., the nucleic acid can be one in which at least one codon, at preferably at least 10%, or 20% of the codons has been altered such that the sequence is optimized for expression in *E. coli*, yeast, human, insect, NS0, or CHO cells.

[00217] In a preferred embodiment, the nucleic acid differs (e.g., differs by substitution, insertion, or deletion) from that of the sequences provided, e.g., as follows: by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the nucleotides in the subject nucleic acid. If necessary for this analysis the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences. The differences are, preferably, differences or changes at nucleotides encoding a non-essential residue(s) or a conservative substitution(s).

[00218] In one embodiment, the first and second nucleic acids are linked, e.g., contained in the same vector. In other embodiments, the first and second nucleic acids are unlinked, e.g., contained in different vectors.

[00219] In another aspect, the invention features host cells and vectors (e.g., recombinant expression vectors) containing the nucleic acids, e.g., the first and second nucleic acids, of the invention.

[00220] Prokaryotic or eukaryotic host cells may be used. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell, but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. A host cell can be any prokaryotic, e.g., bacterial cells such as *E. coli*, or eukaryotic, e.g., insect cells, yeast, or preferably mammalian cells (e.g., cultured cell or a cell line). Other suitable host cells are known to those skilled in the art.

[00221] Preferred mammalian host cells for expressing the anti-PSMA antibodies, or antigen-binding fragments thereof, include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R.J. Kaufman and P.A. Sharp (1982)

Mol. Biol. 159:601-621), lymphocytic cell lines, e.g., NS0 myeloma cells and SP2 cells, COS cells, and a cell from a transgenic animal, e.g., e.g., mammary epithelial cell.

[00222] In another aspect, the invention features a vector, e.g., a recombinant expression vector. The recombinant expression vectors of the invention can be designed for expression of the modified antibodies, or an antigen-binding fragment thereof, in prokaryotic or eukaryotic cells. For example, polypeptides of the invention can be expressed in *E. coli*, insect cells (e.g., using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, (1990) *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

[00223] Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to an antibody encoded therein, usually to the constant region of the recombinant antibody.

[00224] In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that are operatively linked and control the expression of the antibody chain genes in a host cell.

[00225] In an exemplary system for recombinant expression of a modified antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology

techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium.

Pharmaceutical Compositions

[00226] In another aspect, the present invention provides compositions, e.g., pharmaceutically acceptable compositions, which include a modified antibody molecule described herein, formulated together with a pharmaceutically acceptable carrier.

[00227] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, isotonic and absorption delaying agents, and the like that are physiologically compatible. The carrier can be suitable for intravenous, intramuscular, subcutaneous, parenteral, rectal, spinal or epidermal administration (*e.g.*, by injection or infusion).

[00228] The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions. The preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

[00229] The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

[00230] Therapeutic compositions typically should be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high antibody concentration. Sterile injectable solutions can be prepared by incorporating the active compound

(*i.e.*, antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[00231] The modified antibodies and antibody-fragments of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is intravenous injection or infusion. As described in the Examples below, the anti-PSMA antibody can be administered by intravenous infusion at a rate of less than 10 mg/min, preferably less than or equal to 5 mg/min to reach a dose of about 1 to 100 mg/m^2, preferably about 5 to 50 mg/m^2, about 7 to 25 mg/m^2, and more preferably, about 10 mg/m^2. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g., Sustained and Controlled Release Drug Delivery Systems*, J.R. RobiNSOn, ed., Marcel Dekker, Inc., New York, 1978.

[00232] In certain embodiments, an antibody or antibody portion of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral

therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

[00233] Therapeutic compositions can be administered with medical devices known in the art.

[00234] Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[00235] An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 0.1-20 mg/kg, more preferably 1-10 mg/kg. As described in Examples 10 and 12, the anti-PSMA antibody can be administered by intravenous infusion at a rate of less than 10 mg/min, preferably less than or equal to 5 mg/min to reach a dose of about 1 to 100 mg/m^2, preferably about 5 to 50 mg/m^2, about 7 to 25 mg/m^2, and more preferably, about 10 mg/m^2. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[00236] The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the modified antibody or antibody fragment may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the modified antibody or antibody fragment is outweighed by the therapeutically beneficial effects. A "therapeutically effective dosage" preferably inhibits a measurable parameter, e.g., tumor growth rate by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit a measurable parameter, e.g., cancer, can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition *in vitro* by assays known to the skilled practitioner.

[00237] A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[00238] Also within the scope of the invention are kits comprising an anti-PSMA antibody, preferably a modified antibody, or antigen-binding fragment thereof. The kit can include one or more other elements including: instructions for use; other reagents, e.g., a label, a therapeutic agent, or an agent useful for chelating, or otherwise coupling, an antibody to a label or therapeutic agent, or a radioprotective composition; devices or other materials for preparing the antibody for administration; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject. Instructions for use can include instructions for diagnostic applications of the anti-PSMA antibodies (or antigen-binding fragment thereof) to detect PSMA, *in vitro*, e.g., in a sample, e.g., a biopsy or cells from a patient having a cancer or prostatic disorder, or *in vivo*. The instructions can include instructions for therapeutic application including suggested dosages and/or modes of administration, e.g., in a patient with a

cancer or prostatic disorder. Other instructions can include instructions on coupling of the antibody to a chelator, a label or a therapeutic agent, or for purification of a conjugated antibody, e.g., from unreacted conjugation components. As discussed above, the kit can include a label, e.g., any of the labels described herein. As discussed above, the kit can include a therapeutic agent, e.g., a therapeutic agent described herein. The kit can include a reagent useful for chelating or otherwise coupling a label or therapeutic agent to the antibody, e.g., a reagent discussed herein. For example, a macrocyclic chelating agent, preferably 1,4,7,10-tetraazacyclododecane-N, N', N'',N'''-tetraacetic acid (DOTA), can be included. The DOTA can be supplied as a separate component or the DOTA (or other chelator or conjugating agent) can be supplied already coupled to the antibody. Additional coupling agents, e.g., an agent such as N-hydroxysuccinimide (NHS), can be supplied for coupling the chelator, e.g., DOTA, to the antibody. In some applications the antibody will be reacted with other components, e.g., a chelator or a label or therapeutic agent, e.g., a radioisotope, e.g., yttrium or lutetium. In such cases the kit can include one or more of a reaction vessel to carry out the reaction or a separation device, e.g., a chromatographic column, for use in separating the finished product from starting materials or reaction intermediates.

[00239] The kit can further contain at least one additional reagent, such as a diagnostic or therapeutic agent, e.g., a diagnostic or therapeutic agent as described herein, and/or one or more additional anti-PSMA antibodies (or fragments thereof), formulated as appropriate, in one or more separate pharmaceutical preparations.

[00240] The kit can further contain a radioprotectant. The radiolytic nature of isotopes, e.g., ^{90}Yttrium (^{90}Y) is known. In order to overcome this radiolysis, radioprotectants may be included, e.g., in the reaction buffer, as long as such radioprotectants are benign, meaning that they do not inhibit or otherwise adversely affect the labeling reaction, e.g., of an isotope, such as of ^{90}Y, to the antibody.

[00241] The formulation buffer of the present invention may include a radioprotectant such as human serum albumin (HSA) or ascorbate, which minimize radiolysis due to yttrium or other strong radionuclides. Other radioprotectants are known in the art and can also be used in the formulation buffer of the present invention, i.e., free radical scavengers (phenol, suffites, glutathione, cysteine, gentisic acid, nicotinic acid, ascorbyl palmitate, $HOP(:O)H_2I$ glycerol, sodium formaldehyde sulfoxylate, $Na_2S_20.$, $Na_2S_20_3$, and $S0_2$, etc.).

[00242] A preferred kit is one useful for radiolabeling a chelator- conjugated protein or peptide with a therapeutic radioisotope for administration to a patient. The kit includes (i) a vial containing chelator-conjugated antibody, (ii) a vial containing formulation buffer for stabilizing and administering the radiolabeled antibody to a patient, and (iii) instructions for performing the radiolabeling procedure. The kit provides for exposing a chelator-conjugated antibody to the radioisotope or a salt thereof for a sufficient amount of time under amiable conditions, e.g., as recommended in the instructions. A radiolabeled antibody having sufficient purity, specific activity and binding specificity is produced. The radiolabeled antibody may be diluted to an appropriate concentration, e.g., in formulation buffer, and administered directly to the patient with or without further purification. The chelator- conjugated antibody may be supplied in lyophilized form.

Uses of the Invention

[00243] The modified antibodies have *in vitro* and *in vivo* diagnostic, therapeutic and prophylactic utilities. For example, these antibodies can be administered to cells in culture, e.g. *in vitro* or *ex vivo*, or in a subject, e.g., *in vivo*, to treat, prevent, and/or diagnose a variety of disorders, such as cancers (prostatic and non-prostatic cancers), as well as non-cancerous prostatic conditions (e.g., benign hyperplastic prostatic disorders).

[00244] As used herein, the term "subject" is intended to include human and non-human animals. Preferred human animals include a human patient having a disorder characterized by abnormal functioning of a PSMA-expressing cell, e.g., a cancer cell or a prostatic cell. The term "non-human animals" of the invention includes all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, etc.

[00245] In one embodiment, the subject is a human subject. Alternatively, the subject can be a mammal expressing a PSMA-like antigen with which a modified antibody of the invention cross-reacts. A modified antibody molecule of the invention can be administered to a human subject for therapeutic purposes (discussed further below). Moreover, a modified anti-PSMA antibody (or fragment thereof) can be administered to a non-human mammal expressing the PSMA-like antigen with which the modified antibody cross-reacts (e.g., a primate, pig or mouse) for veterinary purposes or as an animal model of human disease. Regarding the latter, such

animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (e.g., testing of dosages and time courses of administration).

Therapeutic Uses

[00246] In one embodiment, the invention provides a method of treating, e.g., ablating or killing, a cell, e.g., a prostatic cell (e.g., a cancerous or non-cancerous prostatic cell, e.g., a normal, benign or hyperplastic prostatic epithelial cell), or a malignant, non-prostatic cell, e.g., cell found in a non-prostatic solid tumor, a soft tissue tumor, or a metastatic lesion (e.g., a cell found in renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), pancreatic (e.g., pancreatic duct) cancer and/or metastasis, melanoma (e.g., malignant melanoma), or soft tissue sarcoma). Methods of the invention include the steps of contacting the cell, or a nearby cell, e.g., a vascular endothelial cell proximate to the cell, with a modified anti-PSMA antibody, e.g., a modified anti-PSMA antibody as described herein, in an amount sufficient to treat, e.g., ablate or kill, the cell.

[00247] The subject method can be used on cells in culture, e.g. *in vitro* or *ex vivo*. For example, prostatic cells (e.g., malignant or normal, benign or hyperplastic prostate epithelial cells) or non-prostatic cancerous or metastatic cells (e.g., renal, an urothelial, colon, rectal, lung, breast or liver, cancerous or metastatic cells) can be cultured *in vitro* in culture medium and the contacting step can be effected by adding the modified anti-PSMA antibody or fragment thereof, to the culture medium. The method can be performed on cells (e.g., prostatic cells, or non-prostatic cancerous or metastatic cells) present in a subject, as part of an *in vivo* (e.g., therapeutic or prophylactic) protocol. For *in vivo* embodiments, the contacting step is effected in a subject and includes administering the modified anti-PSMA antibody or fragment thereof to the subject under conditions effective to permit both binding of the antibody or fragment to the cell, or the vascular endothelial cell proximate to the cell, and the treating, e.g., the killing or ablating of the cell.

[00248] Examples of prostatic disorders that can be treated or prevented include, but are not limited to, genitourinary inflammation (e.g., inflammation of smooth muscle cells) as in prostatitis; benign enlargement, for example, nodular hyperplasia (benign prostatic hypertrophy or

hyperplasia); and cancer, e.g., adenocarcinoma or carcinoma, of the prostate and/or testicular tumors.

[00249] As used herein, the term "cancer" is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness.

[00250] Examples of non-prostatic cancerous disorders include, but are not limited to, solid tumors, soft tissue tumors, and metastatic lesions. Examples of solid tumors include malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting lung, breast, lymphoid, gastrointestinal (e.g., colon), and genitourinary tract (e.g., renal, urothelial cells), pharynx. Adenocarcinomas include malignancies such as most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. Metastatic lesions of the aforementioned cancers can also be treated or prevented using the methods and compositions of the invention.

[00251] The subject method can be useful in treating malignancies of the various organ systems, such as those affecting lung, breast, lymphoid, gastrointestinal (e.g., colon), bladder, genitourinary tract (e.g., prostate), pharynx, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus.

[00252] In other embodiments, the antibodies of the invention can be used for the diagnosis and treatment of a subject experiencing pain or suffering from a pain-associated disorder. Preferably, the subject is a human, e.g., a patient with pain or a pain-associated disorder disclosed herein. For example, the subject could have a disease of the prostate, e.g., benign prostatic hyperplasia or prostate cancer, or non-prostate cancer, e.g., a cancer having vasculature which expresses PSMA (e.g., renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), or pancreatic (e.g., pancreatic duct) cancer, melanoma (e.g., malignant melanoma), or soft tissue sarcoma). The pain can be associated with bones, as well as with obstructive voiding symptoms due to enlarged prostate, e.g., urinary hesitancy or diminished urinary stream, frequency or nocturia. The treatment of pain using the modified anti-PSMA

antibodies of the invention can lead to a decreased or dramatically lowered need, or even eliminate the need, for analgesics, e.g., narcotics. In addition, by reducing pain, the methods of treatment can restore the mobility of, e.g., limbs, that have become dysfunctional as a result of pain associated with movement.

[00253] Methods of administering modified antibody molecules are described above. Suitable dosages of the molecules used will depend on the age and weight of the subject and the particular drug used. The modified antibody molecules can be used as competitive agents for ligand binding to inhibit, reduce an undesirable interaction.

[00254] In one embodiment, the anti-PSMA antibodies, e.g., the modified anti-PSMA antibodies, or antigen-binding fragments thereof, can be used to kill or ablate cancerous cells and normal, benign hyperplastic, and cancerous prostate epithelial cells *in vivo*. For example, the anti-PSMA antibodies can be used to treat or prevent a disorder described herein. The antibodies, e.g., the modified antibodies, (or fragments thereof) can be used by themselves or conjugated to a second agent, e.g., a cytotoxic drug, radioisotope, or a protein, e.g., a protein toxin or a viral protein. This method includes: administering the modified antibody, alone or conjugated to a cytotoxic drug, to a subject requiring such treatment.

[00255] Since the anti-PSMA antibodies (or fragments thereof) recognize normal, benign hyperplastic, and cancerous prostate epithelial cells, any such cells to which the modified antibodies bind are destroyed. Although such administration may destroy normal prostate epithelial cells, this is not problematic, because the prostate is not required for life or survival. Although the prostate may indirectly contribute to fertility, this is not likely to be a practical consideration in patients receiving the treatment of the present invention. In the case of cancerous tissues, since the modified antibodies recognize vascular endothelial cells that are proximate to cancerous cells, binding of the modified antibody/cytotoxic drug complex to these vascular endothelial cells destroys them, thereby cutting off the blood flow to the proximate cancerous cells and, thus, killing or ablating these cancerous cells. Alternatively, the modified antibodies, by virtue of their binding to vascular endothelial cells that are proximate to cancerous cells, are localized proximate to the cancerous cells. Thus, by use of suitable modified antibodies (including those containing substances effective to kill cells nondiscriminatingly but only over a short range), cells in cancerous tissue (including cancerous cells) can be selectively killed or ablated.

[00256] The antibodies of the present invention may be used to deliver a variety of therapeutic agents, e.g., a cytotoxic moiety, e.g., a therapeutic drug, a radioisotope, molecules of plant, fungal, or bacterial origin, or biological proteins (e.g., protein toxins) or particles (e.g., a recombinant viral particles, e.g., via a viral coat protein), or mixtures thereof. The therapeutic agent can be an intracellularly active drug or other agent, such as short-range radiation emitters, including, for example, short-range, high-energy α-emitters, as described herein. In some preferred embodiments, the anti-PSMA antibody, or antigen binding fragment thereof, can be coupled to a molecule of plant or bacterial origin (or derivative thereof), e.g., a maytansinoid (e.g., maytansinol or the DM1 maytansinoid, see Figure 15). DM1 is a sulfhydryl-containing derivative of maytansine that can be linked to antibodies via a disulfide linker that releases DM1 when inside target cells. The disulfide linkers display greater stability in storage and in serum than other linkers. Maytansine is a cytotoxic agent that effects cell killing by preventing the formation of microtubules and depolymerization of extant microtubules. It is 100- to 1000-fold more cytotoxic than anticancer agents such as doxorubicin, methotrexate, and vinca alkyloid, which are currently in clinical use. Alternatively, the anti-PSMA antibody, or antigen binding fragment thereof, can be coupled to a taxane, a calicheamicin, a proteosome inhibitor, or a topoisomerase inhibitor. [(1R)-3-methyl-1-[[(2S)-1-oxo-3-phenyl-2-[(3-mercaptoacetyl) amino]propyl]amino]butyl] Boronic acid is a suitable proteosome inhibitor. N,N'-bis[2-(9-methylphenazine-1-carboxamido)ethyl]-1,2-ethanediamine is a sutiable topoisomerase inhibitor.

[00257] Enzymatically active toxins and fragments thereof are exemplified by diphtheria toxin A fragment, nonbinding active fragments of diphtheria toxin, exotoxin A (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, α-sacrin, certain *Aleurites fordii* proteins, certain Dianthin proteins, *Phytolacca americana* proteins (PAP, PAPII and PAP-S), *Morodica charantia* inhibitor, curcin, crotin, *Saponaria officinalis* inhibitor, gelonin, mitogillin, restrictocin, phenomycin, and enomycin. In a preferred embodiment, the anti-PSMA antibody is conjugated to maytansinoids, *e.g.*, maytansinol (see US Patent No. 5,208,020), CC-1065 (see US Patent Nos. 5,475,092, 5,585,499, 5,846,545). Procedures for preparing enzymatically active polypeptides of the immunotoxins are described in W084/03508 and W085/03508, which are hereby incorporated by reference, and in the appended Examples below. Examples of cytotoxic moieties that can be conjugated to the antibodies include adriamycin, chlorambucil, daunomycin, methotrexate, neocarzinostatin, and platinum.

[00258] To kill or ablate normal, benign hyperplastic, and cancerous prostate epithelial cells, a first antibody, e.g., a modified antibody, can be conjugated with a prodrug which is activated only when in close proximity with a prodrug activator. The prodrug activator is conjugated with a second antibody, e.g., a second modified antibody according to the present invention, preferably one that binds to a non-competing site on the prostate specific membrane antigen molecule. Whether two modified antibodies bind to competing or non-competing binding sites can be determined by conventional competitive binding assays. For example, monoclonal antibodies J591, J533, and E99 bind to competing binding sites on the prostate specific membrane antigen molecule. Monoclonal antibody J415, on the other hand, binds to a binding site that is non-competing with the site to which J591, J533, and E99 bind. Thus, for example, the first modified antibody can be one of J591, J533, and E99, and the second modified antibody can be J415. Alternatively, the first modified antibody can be J415, and the second modified antibody can be one of J591, J533, and E99. Drug-prodrug pairs suitable for use in the practice of the present invention are described in Blakely et al., "ZD2767, an Improved System for Antibody-directed Enzyme Prodrug Therapy That Results in Tumor Regressions in Colorectal Tumor Xenografts," (1996) *Cancer Research*, 56:3287-3292, which is hereby incorporated by reference.

[00259] Alternatively, the antibody, e.g., the modified antibody, can be coupled to high energy radiation emitters, for example, a radioisotope, such as ^{131}I, a γ-emitter, which, when localized at the tumor site, results in a killing of several cell diameters. See, e.g., S.E. Order, "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy", *Monoclonal Antibodies for Cancer Detection and Therapy*, R.W. Baldwin et al. (eds.), pp 303-316 (Academic Press 1985), which is hereby incorporated by reference. Other suitable radioisotopes include α-emitters, such as ^{212}Bi, ^{213}Bi, and ^{211}At, and β-emitters, such as ^{186}Re and ^{90}Y. Radiotherapy is expected to be particularly effective, because prostate epithelial cells and vascular endothelial cells within cancers are relatively radiosensitive. Moreover, Lu^{117} may also be used as both an imaging and cytotoxic agent.

[00260] Radioimmunotherapy (RIT) using antibodies labeled with ^{131}I, ^{90}Y, and ^{177}Lu is under intense clinical investigation. There are significant differences in the physical characteristics of these three nuclides and as a result, the choice of radionuclide can be important in order to deliver maximum radiation dose to the tumor. The higher beta energy particles of ^{90}Y

may be good for bulky tumors, but it may not be necessary for small tumors and especially bone metastases, (e.g. those common to prostate cancer). The relatively low energy beta particles of ^{131}I are ideal, but *in vivo* dehalogenation of radioiodinated molecules is a major disadvantage for internalizing antibody. In contrast, ^{177}Lu has low energy beta particle with only 0.2-0.3 mm range and delivers much lower radiation dose to bone marrow compared to ^{90}Y. In addition, due to longer physical half-life (compared to ^{90}Y), the tumor residence times are higher. As a result, higher activities (more mCi amounts) of ^{177}Lu labeled agents can be administered with comparatively less radiation dose to marrow. There have been several clinical studies investigating the use of ^{177}Lu labeled antibodies in the treatment of various cancers. (Mulligan T et al. (1995) *Clin Cancer Res.* 1: 1447-1454; Meredith RF, et al. (1996) *J Nucl Med* 37:1491-1496; Alvarez RD, et al. (1997) *Gynecologic Oncology* 65: 94-101).

[00261] The antibodies of the invention can also be conjugated or fused to viral surface proteins present on viral particles. For example, a single-chain anti-PSMA antibody of the present invention could be fused (e.g., to form a fusion protein) to a viral surface protein. Alternatively, a whole anti-PSMA antibody of the present invention, or a fragment thereof, could be chemically conjugated (e.g., via a chemical linker) to a viral surface protein. Preferably, the virus is one that fuses with endocytic membranes, e.g., an influenza virus, such that the virus is internalized along with the anti-PSMA antibody and thereby infects PSMA-expressing cells. The virus can be genetically engineered as a cellular toxin. For example, the virus could express or induce the expression of genes that are toxic to cells, e.g., cell death promoting genes. Preferably, such viruses would be incapable of viral replication.

[00262] The antibodies, e.g., the modified antibodies of the invention, can be used directly *in vivo* to eliminate antigen-expressing cells via natural complement or antibody-dependent cellular cytotoxicity (ADCC). Modified antibody molecules of the invention, which have complement binding sites, such as portions from IgG1, -2, or -3 or IgM which bind complement can also be used in the presence of complement. In one embodiment, *ex vivo* treatment of a population of cells comprising target cells with a binding agent of the invention and appropriate effector cells can be supplemented by the addition of complement or serum containing complement. Phagocytosis of target cells coated with modified antibodies or fragments thereof of the invention can be improved by binding of complement proteins. In another embodiment

target cells coated with the modified antibodies or fragments thereof can also be lysed by complement.

[00263] The antibodies, e.g., the modified antibodies, of the present invention can be used and sold together with equipment, as a kit, to detect the particular label.

[00264] Also encompassed by the present invention is a method of killing or ablating cells which involves using the antibodies described herein, e.g., the modified antibodies for preventing a PSMA-related disorder. For example, these materials can be used to prevent or delay development or progression of prostate or other cancers.

[00265] Use of the therapeutic methods of the present invention to treat prostate and other cancers has a number of benefits. Since the modified antibodies according to the present invention only target cancerous cells (such as cells of cancerous tissues containing vascular endothelial cells) and prostate epithelial cells, other tissue is spared. As a result, treatment with such modified antibodies is safer, particularly for elderly patients. Treatment according to the present invention is expected to be particularly effective, because it directs high levels of modified antibodies, such as antibodies or binding portions thereof, probes, or ligands, to the bone marrow and lymph nodes where prostate cancer metastases and metastases of many other cancers predominate. Moreover, the methods of the present invention are particularly well-suited for treating prostate cancer, because tumor sites for prostate cancer tend to be small in size and, therefore, easily destroyed by cytotoxic agents. Treatment in accordance with the present invention can be effectively monitored with clinical parameters, such as, in the case of prostate cancer, serum prostate specific antigen and/or pathological features of a patient's cancer, including stage, Gleason score, extracapsular, seminal, vesicle or perineural invasion, positive margins, involved lymph nodes, disease related pain, e.g., bone pain, etc. Alternatively, these parameters can be used to indicate when such treatment should be employed.

[00266] The invention also features methods of treating pain, e.g., reducing pain, experienced by a subject having or diagnosed with prostate disease, e.g., benign prostatic hyperplasia or prostate cancer, or non-prostate cancer, e.g., a cancer having vasculature which expresses PSMA (e.g., renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), or pancreatic (e.g., pancreatic duct) cancer, melanoma (e.g., malignant melanoma), or soft tissue sarcoma). The methods include administering an anti-PSMA antibody as described herein, e.g.,

a modified anti-PSMA antibody, to a subject in an amount sufficient to treat, e.g., reduce, the pain associated with prostate disease or non-prostate cancer. The subject may have no signs of prostate disease or non-prostate cancer other than, e.g., elevated levels of serum PSA and the sensation of pain. Patients that have prostate cancer often experience bone pain, as well as, pain associated with obstructive voiding symptoms due to enlarged prostate, e.g., urinary hesitancy or diminished urinary stream, frequency or nocturia. The treatment of pain using the modified anti-PSMA antibodies of the invention can lead to a decreased or dramatically lowered need, or even eliminate the need, for analgesics, e.g., narcotics. By reducing pain, the methods of treatment can restore the mobility of, e.g., limbs, that have become dysfunctional as a result of pain associated with movement.

[00267] Because the antibodies, e.g., the modified antibodies, of the present invention bind to living prostate cells, therapeutic methods for treating prostate cancer using these modified antibodies are not dependent on the presence of lysed prostate cells. For the same reasons, diagnostic and imaging methods which determine the location of living normal, benign hyperplastic, or cancerous prostate epithelial cells (as well as vascular endothelial cells within cancers) are much improved by employing the modified antibodies of the present invention. In addition, the ability to differentiate between living and dead prostate cells can be advantageous, especially to monitor the effectiveness of a particular treatment regimen.

[00268] The antibodies, e.g., the modified antibodies, or antigen-binding portions thereof, of the present invention bind to extracellular domains of prostate specific membrane antigens or portions thereof in normal, benign hyperplastic, and cancerous prostate epithelial cells as well as vascular endothelial cells proximate to cancerous cells. As a result, when practicing the methods of the present invention to kill, ablate, or detect normal, benign hyperplastic, and cancerous prostate epithelial cells as well as vascular endothelial cells proximate to cancerous cells, the antibodies, e.g., the modified antibodies, bind to all such cells, not only to cells which are fixed or cells whose intracellular antigenic domains are otherwise exposed to the extracellular environment. Consequently, binding of the antibodies, e.g., the modified antibodies, is concentrated in areas where there are prostate epithelial cells, irrespective of whether these cells are fixed or unfixed, viable or necrotic. Additionally or alternatively, these antibodies, e.g., these modified antibodies, or binding portions thereof, bind to and are internalized with prostate

[00271] Anti-PSMA antibodies of the invention can be administered in combination with one or more of the existing modalities for treating prostate cancers, including, but not limited to: surgery (e.g., radical prostatectomy); radiation therapy (e.g., external-beam therapy which involves three dimensional, conformal radiation therapy where the field of radiation is designed to conform to the volume of tissue treated; interstitial-radiation therapy where seeds of radioactive compounds are implanted using ultrasound guidance; and a combination of external-beam therapy and interstitial-radiation therapy); hormonal therapy, which can be administered before or following radical prostatectomy or radiation (e.g., treatments which reduce serum testosterone concentrations, or inhibit testosterone activity, e.g., administering a leuteinizing hormone-releasing hormone (LHRH) analog or agonist (e.g., Lupron, Zoladex, leuprolide, buserelin, or goserelin) or antagonists (e.g., Abarelix). Non-steroidal anti-androgens, e.g., flutamide, bicalutimade, or nilutamide, can also be used in hormonal therapy, as well as steroidal anti-androgens (e.g., cyproterone acetate or megastrol acetate), estrogens (e.g., diethylstilbestrol), PROSCAR™, secondary or tertiary hormonal manipulations (e.g., involving corticosteroids (e.g., hydrocortisone, prednisone, or dexamethasone), ketoconazole, and/or aminogluthethimide), inhibitors of 5a-reductase (e.g., finisteride), herbal preparations (e.g., PC-SPES), hypophysectomy, and adrenalectomy. Furthermore, hormonal therapy can be performed intermittently or using combinations of any of the above treatments, e.g., combinded use of leuprolide and flutamide.

[00272] In other embodiments, the anti-PSMA antibodies, e.g., the modified anti-PSMA antibodies, are administered in combination with an immunomodulatory agent, e.g., IL-1, 24, 6, or 12, or interferon alpha or gamma. As described in Example 14 below, the combination of antibodies having a human constant regions and IL-2 potentially is expected to enhance the efficacy of the monoclonal antibody. IL-2 will function to augment the reticuloendothelial system to recognize antigen-antibody complexes by its effects on NK cells and macrophages. Thus, by stimulating NK cells to release IFN, GM-CSF, and TNF, these cytokines will increase the cell surface density of Fc receptors, as well as the phagocytic capacities of these cells. Therefore, the effector arm of both the humoral and cellular arms will be artificially enhanced. The net effect will be to improve the efficiency of monoclonal antibody therapy, so that a maximal response may be obtained. A small number of clinical trials have combined IL-2 with a monoclonal antibody (Albertini et al. (1997) *Clin Cancer Res* 3:1277-1288; Frost et al. (1997)

Cancer 80:317-333; Kossman et al. (1999) *Clin Cancer Res* 5:2748-2755). IL-2 can be administered by either bolus or continuous infusion. Accordingly, the antibodies of the invention can be administered in combination with IL-2 to maximize their therapeutic potential.

Diagnostic Uses

[00273] In one aspect, the present invention provides a diagnostic method for detecting the presence of a PSMA protein *in vitro* (e.g., in a biological sample, such as a tissue biopsy, e.g., from a cancerous or prostatic tissue) or *in vivo* (e.g., *in vivo* imaging in a subject). The method includes: (i) contacting the sample with a modified anti-PSMA antibody or fragment thereof, or administering to the subject, the modified anti-PSMA antibody; (optionally) (ii) contacting a reference sample, e.g., a control sample (e.g., a control biological sample, such as plasma, tissue, biopsy) or a control subject)); and (iii) detecting formation of a complex between the anti-PSMA antibody, and the sample or subject, or the control sample or subject, wherein a change, e.g., a statistically significant change, in the formation of the complex in the sample or subject relative to the control sample or subject is indicative of the presence of PSMA in the sample.

[00274] Preferably, the anti-PSMA antibody (or fragment thereof) is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials, as described above and described in more detail below.

[00275] Complex formation between the anti-PSMA antibody and PSMA can be detected by measuring or visualizing either the antibody (or antibody fragment) bound to the PSMA antigen or unbound antibody (or antibody fragment). Conventional detection assays can be used, e.g., an enzyme-linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. Alternative to labeling the anti-PSMA antibody, the presence of PSMA can be assayed in a sample by a competition immunoassay utilizing standards labeled with a detectable substance and an unlabeled anti-PSMA antibody. In this assay, the biological sample, the labeled standards and the PSMA binding agent are combined and the amount of labeled standard bound to the unlabeled antibody is determined. The amount of PSMA in the sample is inversely proportional to the amount of labeled standard bound to the PSMA binding agent.

[00276] In still another embodiment, the invention provides a method for detecting the presence of PSMA-expressing cancerous tissues (particularly the vascular endothelial cells therein) and normal, benign hyperplastic, and cancerous prostate epithelial cells *in vivo*. The method includes (i) administering to a subject (e.g., a patient having a cancer or prostatic disorder) an anti-PSMA antibody,preferably a modified antibody, conjugated to a detectable marker; (ii) exposing the subject to a means for detecting said detectable marker to the PSMA-expressing tissues or cells. Particularly preferred antibodies include modified antibodies having CDRs from any of a J591, J415, J533 or E99, and in particular deimmunized versions of these antibodies, particularly deJ591 or deJ415.

[00277] Examples of labels useful for diagnostic imaging in accordance with the present invention are radiolabels such as ^{131}I, ^{111}In, ^{123}I, ^{99m}Tc, ^{32}P, ^{125}I, ^{3}H, ^{14}C, and ^{188}Rh, fluorescent labels such as fluorescein and rhodamine, nuclear magnetic resonance active labels, positron emitting isotopes detectable by a positron emission tomography ("PET") scanner, chemiluminescers such as luciferin, and enzymatic markers such as peroxidase or phosphatase. Short-range radiation emitters, such as isotopes detectable by short-range detector probes, such as a transrectal probe, can also be employed. These isotopes and transrectal detector probes, when used in combination, are especially useful in detecting prostatic fossa recurrences and pelvic nodal disease. The modified antibody can be labeled with such reagents using techniques known in the art. For example, see Wensel and Meares (1983) *Radioimmunoimaging and Radioimmunotherapy*, Elsevier, New York, which is hereby incorporated by reference, for techniques relating to the radiolabeling of antibodies. See also, D. Colcher et al. (1986) *Meth. Enzymol.* 121: 802-816, which is hereby incorporated by reference.

[00278] In the case of a radiolabeled modified antibody, the modified antibody is administered to the patient, is localized to the tumor bearing the antigen with which the modified antibody reacts, and is detected or "imaged" *in vivo* using known techniques such as radionuclear scanning using e.g., a gamma camera or emission tomography. See e.g., A.R. Bradwell et al., "Developments in Antibody Imaging", *Monoclonal Antibodies for Cancer Detection and Therapy*, R.W. Baldwin et al., (eds.), pp 65-85 (Academic Press 1985), which is hereby incorporated by reference. Alternatively, a positron emission transaxial tomography scanner, such as designated Pet VI located at Brookhaven National Laboratory, can be used where the radiolabel emits positrons (e.g., ^{11}C, ^{18}F, ^{15}O, and ^{13}N).

[00279] Fluorophore and chromophore labeled modified antibodies can be prepared from standard moieties known in the art. Since antibodies and other proteins absorb light having wavelengths up to about 310 nm, the fluorescent moieties should be selected to have substantial absorption at wavelengths above 310 nm and preferably above 400 nm. A variety of suitable fluorescent compounds and chromophores are described by Stryer (1968) *Science*, 162:526 and Brand, L. et al. (1972) *Annual Review of Biochemistry*, 41:843-868, which are hereby incorporated by reference. The modified antibodies can be labeled with fluorescent chromophore groups by conventional procedures such as those disclosed in U.S. Patent Nos. 3,940,475, 4,289,747, and 4,376,110, which are hereby incorporated by reference.

[00280] One group of fluorescers having a number of the desirable properties described above is the xanthene dyes, which include the fluoresceins derived from 3,6-dihydroxy-9-henylxanthhydrol and resamines and rhodamines derived from 3,6-diamino-9-phenylxanthydrol and lissanime rhodamine B. The rhodamine and fluorescein derivatives of 9-o-carboxyphenylxanthhydrol have a 9-o-carboxyphenyl group. Fluorescein compounds having reactive coupling groups such as amino and isothiocyanate groups such as fluorescein isothiocyanate and fluorescamine are readily available. Another group of fluorescent compounds are the naphthylamines, having an amino group in the α or β position.

[00281] In cases where it is important to distinguish between regions containing live and dead prostate epithelial cells or to distinguish between live and dead prostate epithelial cells, the antibodies of the present invention (or other modified antibodies of the present invention), labeled as described above, can be coadministered along with an antibody or other modified antibody which recognizes only living or only dead prostate epithelial cells labeled with a label which can be distinguished from the label used to label the subject antibody. By monitoring the concentration of the two labels at various locations or times, spatial and temporal concentration variations of living and dead normal, benign hyperplastic, and cancerous prostate epithelial cells can be ascertained. In particular, this method can be carried out using the labeled antibodies of the present invention, which recognize both living and dead epithelial prostate cells, and labeled 7E11 antibodies (Horoszewicz et al. (1987) *Anticancer Research* 7:927-936), which recognize only dead epithelial prostate cells.

[00282] In other embodiments, the invention provide methods for determining the dose, e.g., radiation dose, that different tissues are exposed to when a subject, e.g., a human subject, is

administered an anti-PSMA antibody that is conjugated to a radioactive isotope. The method includes: (i) administering an anti-PSMA antibody as described herein, e.g., a modified anti-PSMA antibody, that is labeled with a radioactive isotope to a subject; (ii) measuring the amount of radioactive isotope located in different tissues, e.g., prostate, liver, kidney, or blood, at various time points until some or all of the radioactive isotope has been eliminated from the body of the subject; and (iii) caluculating the total dose of radiation received by each tissue analyzed. The measurements can be taken at scheduled time points, e.g., day 1, 2, 3, 5, 7, and 12, following administration (at day 0) of the radioactively labeled anti-PSMA antibody to the subject. The concentration of radioisotope present in a given tissue, integrated over time, and multiplied by the specific activity of the radioisotope can be used to calculate the dose that a given tissue receives. Pharmacological information generated using anti-PSMA antibodies labeled with one radioactive isotope, e.g., a gamma-emitter, e.g., ^{111}In, can be used to calculate the expected dose that the same tissue would receive from a different radioactive isotope which cannot be easily measured, e.g., a beta-emitter, e.g., ^{90}Y.

Pharmacogenomics

[00283] With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23:983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43:254-266. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype," or "drug response genotype.") Thus, another aspect of the

invention provides methods for tailoring an individual's prophylactic or therapeutic treatment according to that individual's drug response genotype.

[00284] Information generated from pharmacogenomic research can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when administering a therapeutic composition, e.g., a composition consisting of one or more anti-PSMA antibodies, or derivatized form(s) thereof, to a patient, as a means of treating a disorder, e.g., a cancer or prostatic disorder as described herein.

[00285] In one embodiment, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies when determining whether to administer a pharmaceutical composition, e.g., a composition consisting of one or more anti-PSMA antibodies, derivatized form(s) thereof, and optionally a second agent, to a subject. In another embodiment, a physician or clinician may consider applying such knowledge when determining the dosage, e.g., amount per treatment or frequency of treatments, of a pharmaceutical composition, e.g., a pharmaceutical composition as described herein, administered to a patient.

[00286] In yet another embodiment, a physician or clinician may determine the genotypes, at one or more genetic loci, of a group of subjects participating in a clinical trial, wherein the subjects display a disorder, e.g., a cancer or prostatic disorder as described herein, and the clinical trial is designed to test the efficacy of a pharmaceutical composition, e.g., a composition consisting of one or more anti-PSMA antibodies, and optionally a second agent, and wherein the physician or clinician attempts to correlate the genotypes of the subjects with their response to the pharmaceutical composition.

Deposits

[00287] Hybridomas E99, J415, J533, and J591 have been deposited pursuant to, and in satisfaction of, the requirements of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure with the American Type Culture Collection ("A.T.C.C.") at 10801 University Boulevard, Manassas, VA 20110-2209. Hybridoma E99 was deposited on May 2, 1996, and received A.T.C.C. Designation Number HB-12101. Hybridoma J415 was deposited on May 30, 1996, and received A.T.C.C.

Designation Number HB-12109. Hybridomas J533 and J591 were deposited on June 6, 1996, and received A.T.C.C. Designation Numbers HB-12127 and HB-12126, respectively.

[00288] An NS0 cell line producing deimmunized J591 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on September 18, 2001 and assigned Accession Number PTA-3709. An NS0 cell line producing deimmunized J415 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on March 21, 2002 and assigned Accession Number PTA-4174. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

[00289] The following invention is further illustrated by the following examples, which should not be construed as further limiting. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference.

EXAMPLES

Example 1 - Chelation Of Anti-PSMA Antibodies To ^{111}Indium, ^{90}Yttrium, and ^{177}Lutetium

[00290] The modified anti-PSMA monoclonal antibodies can be radiolabeled with ^{111}Indium, ^{90}Yttrium, or ^{177}Lutetium by directly coupling one of the four carboxylic acid groups of the chelator 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) to primary amines present on the surface of the antibodies. The DOTA conjugated antibody is then purified, sterile filtered, and vialed. Prior to use, the purified antibodies can be mixed with the desired radiolabel which binds to DOTA.

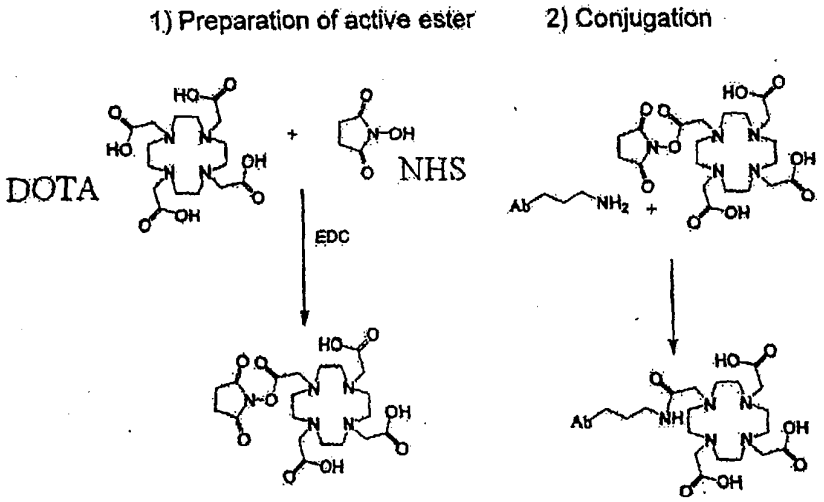
Chelation Process

[00291] Monoclonal antibody deJ591 was conjugated with 1,4,7,10-tetraazacyclododecane-N,N', N", N"'-tetraacetic acid (DOTA) and subsequently radiolabeled with ^{111}In, ^{90}Y and ^{177}Lu. Radiolabeling and quality control tests were performed on three separate vials of clinical grade mAb deJ591.

[00292] All reagents used in the conjugation and purification of deJ591 were made from pyrogen-free water. In the specific case of NH_4OAC buffer and sodium phosphate buffer, the solutions were purified with Chelex 100 (Bio-Rad, CA) to remove any metal ions.

Conjugation of Antibody with 1,4,7,10-Tetraazacyclododecane-N,N', N", N"'-tetraacetic acid (DOTA)

[00293] The monoclonal antibody deJ591 was modified with 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) as follows. Briefly, 25 mg of deJ591 was concentrated in a 30 kDa microsep centrifugal concentrator (Pall Filtron, MA) and washed with 5 x 4 mL of 1% DTPA (pH 5.0), over a period of 24 hours. The antibody buffer was then changed to 0.1 M phosphate (pH 7.0) using the same centrifugal technique. An active ester of DOTA was created by dissolving 146 mg DOTA (0.361 mmoles) and 36 mg N-hydroxysuccinimide (0.313 mmoles) in 2 ml of water and adjusting the pH to 7.3 with NaOH, prior to the addition of 10 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (see below).



This reaction mixture was cooled on ice for 1 hour before being added to the deJ591 solution. The resultant DOTA-deJ591 was separated from the excess DOTA and other reactants by repeated washing with 0.3 M NH_4OAc (20 x 4 mL) and centrifugal concentration. The purified conjugate was then sterilized by filtration through a 0.22 μm filter and stored in a sterile polypropylene vial at 4°C.

[00294] The concentration of the DOTA-deJ591 conjugate was assayed by determining the UV absorption at 280 nm and two 50 μL aliquots mixed with either 20 or 30 μL of a 1.30 mM solution of $InCl_3$ (0.01 M HCl) spiked with a tracer amount of ^{111}In. The mixture is incubated at 37°C for 16 hours and then analyzed by ITLC, using silica gel impregnated glass fiber 10 cm strip (ITLC-SG, Gelman, prod. # 61885) and an eluant of 1% DTPA (pH 6.0). The antibody bound activity remains at the origin and free ^{111}In moves with the solvent front as an ^{111}In-DTPA complex. The relative amounts of ^{111}In and ^{111}In -DOTA-J591 is determined by cutting the ITLC strip at a Rf of 0.5 and counting the two halves with a Na(Tl)I detector. The number of binding sites is calculated by considering the molar reaction ratio between ^{111}In and DOTA-deJ591 and the observed ratio of ^{111}In and ^{111}In-DOTA-J591 detected. Typically, 5.1 molecules of DOTA are conjugated to deJ591. Table 10 shows the results from two conjugations of deJ591.

Table 10: Calculation of the Mean Number of DOTA Molecules Conjugated to deJ591

Test number	**Known ^{111}In/DOTA-J591**	**Observed ^{111}In/DOTA-J591**	**Mean number of DOTA mols per mAb**
	Reaction ratio	TLC ratio	
A	11.76	1.338	5.03
B	17.64	2.469	5.09

Radiolabeling

[00295] The following radiolabeling procedure is described for ^{111}In, but may be used with other radiolabels such as ^{90}Y or ^{177}Lu. Radiolabeling was achieved by adding the ^{111}In (in dilute HCl) to the ammonium acetate buffered DOTA-deJ591. To avoid the effects of autoradiolysis on the antibody, the reaction time was minimized and the reaction mixture purified with a size exclusion column prior to administration. Briefly, a mixture composed of 20 μL of $^{111}InCl_3$ (8 mCi, 0.01 M HCl, 400 μL DOTA-deJ591 (4 mg/ml, 0.3 M NH_4OAc, pH 7) was allowed to react at 37°C for 20 minutes. The reaction mixture was then separated on a 16 mL Biogel-P6DG column (Bio-Rad, CA) equilibrated with 4 x 10 mL of sterile 1% HSA in PBS (HSA meets specification for US licensed albumin; manufactured by Central Laboratory Blood Transfusion Service Swiss Red Cross, Bern, Switzerland, License No. 647). Once the reaction mixture was loaded onto the column, it was washed with a further 2 mL of 1% HSA PBS, before

the main ^{111}In-DOTA-deJ591 fraction was eluted with 5 mL of 1% HSA PBS. The purified ^{111}In-DOTA-deJ591 was then sterile filtered into a sterile evacuated vial. Using this method, specific activity of 7.6 mCi ^{111}In/mg DOTA-deJ591 was achieved.

Alternative Radiolabeling Procedure for ^{111}In

[00296] The following radiolabeling procedure can be used for the routine preparation of ^{111}In-DOTA-J591 for clinical studies and stability studies. Radiolableing is achieved by the addition of ^{111}In chloride and Ammonium acetate buffer (1 M) to DOTA-J591 solution (8 mg/ml, 0.3 M Ammonium acetate, pH 7). To avoid the effects of autoradiolysis on the antibody, the reaction time has been minimized. The labeled ^{111}In-DOTA-J591 is purified using a size exclusion column and sterile filtered using a 0.2m millipore membrane filter prior to administration to patients.

[00297] Briely, ammonium acetate, (10 μL for each mCi of ^{111}In) is added to a reaction vial containing ^{111}In-chloride solution. Subsequently, the DOTA-J591 solution (30 mL or 0.24 mg for each mCi of ^{111}In) is added to the reaction vial and the mixure is gently mixed and incubated at 37°C for 20-30 min. An aliquot of the mixure is tested to determine labeling efficiency using ITLC (SG and 5 mM DTPA, pH 5). If the binding is optimal (>70%), the reaction is stopped by the addition of 10-40 mL of 5 mM DTPA.

[00298] In order to separate or purify ^{111}In-DOTA-J591 from free ^{111}In, the reaction mixture is applied on a Biogel-P6DG column (Bio-Rad, CA), prewashed with 4 x 10 ml of PBS containing 1% Human Serum Albumin (meets specification for US licensed albumin; manufactured by Central Laboratory Blood Transfusion Service Swiss Red Cross, Bern, Switzerland, License No. 647). The ^{111}In-DOTA-J591 is eluted from the column using PBS with 1% HSA and the fractions containing the labeled antibody (typically 5-8 ml) are collected into a sterile container. Following determination of radiochemical purity using ITLC (as before), and if the labeling efficiency is >95%, the labeled complex is filtered into a sterile vial using 0.2m Filter. The final specific activity is typically 3-5 mCi/mg of antibody.

Radiolabeling with ^{90}Y

[00299] The procedure is identical to the procedure described above for ^{111}In, except the incubation time is 10-15 min. Radiochemical purity of 90Y-DOTA-J591 must be >97%.

Radiolabeling with ^{177}Lu

[00300] The procedure is similar to the procedure described above except for two changes. The amount of Ammonium acetate added is reduced (3-5 mL for each mCi of ^{177}Lu) and the incubation time is only 5 min. Radiochemical purity of ^{177}Lu-DOTA-J591 should be >97%.

Radiochemical Purity

[00301] The amount of free ^{111}In in radiolabeled DOTA-deJ591 preparations was evaluated using the instant thin layer chromatography method with a silica gel impregnated glass fiber support and a mobile phase of 1% DTPA (pH 5.5). Briefly, a portion of the radiolabeled DOTA-deJ591 was spotted on a 10 cm ITLC-SG strip (Gelman, prod. # 61885) and developed in 1% DTPA (pH 5.5). Once the solvent front had reached the end of the strip, it was removed from the solvent and cut at a R_f of 0.5. The two portions were assayed for radioactivity and the radiochemical purity determined using the following equation:

Radiochemical purity = (Activity in between R_f 0 and 0.5)/(Total activity in strip)

Immunoreactivity

[00302] The immunoreactivity of the ^{111}In-DOTA-deJ591 preparations was assessed according to the method of Lindmo (Lindmo T. et al. (1994) *J. Immunol. Methods*, 72:77-89, 1994) that extrapolates the binding of the radiolabeled antibody at an infinite excess of antigen. Briefly, five test solutions were prepared (in duplicate) containing 10,000 cpm of ^{111}In-DOTA-deJ591 and various amounts of LNCaP cells, in a total test volume of 250 µL of 0.2 % BSA 10 mM HEPES. The solutions were incubated at 4°C for 60 minutes prior to being isolated (by centrifugation) and washed with ice cold PBS. The membranes were then counted in a gamma counter with standards representing the total radioactivity added. The data was plotted using the Lindmo method as the reciprocal of the substrate concentration (x-axis) against the reciprocal of the fraction bound (y-axis). The data was then fitted according to a least squares linear regression method (Sigma Plot) and the y intercept taken as the reciprocal of the immunoreactivity. A similar method using membranes derived from LNCaP cells, and subsequent centrifugation

isolation of the membranes, gave similar results. The results gave an average immunoreactivity of 72% (see Table 11).

Immunohistochemistry

[00303] Immunohistochemistry was performed on the DOTA conjugated, partially purified, bulk intermediate deJ591. The results showed that the preparation was specific to prostate tissue and the reactivity was equivalent to the naked deJ591 antibody.

Sterility

[00304] The sterility of ^{111}In-DOTA-deJ591 preparations was determined using thioglycollate medium according to the method of USP 24/NF 19. Briefly, quadruplicate 0.1 mL samples of the ^{111}In-DOTA-deJ591 preparations were transferred to 15 mL of fluid thioglycollate medium and the mixture incubated at 35°C for 14 days. The media were visually inspected on the 4th, 7th and 14 days of any signs of growth. All three preparations showed no growth (See Table 11).

Endotoxin

[00305] The endotoxin of ^{111}In-DOTA-deJ591 preparations was determined using the Limulus amebocyte lysate assay according to the USP 24/NF 19. Briefly, a Limulus amebocyte lysate kit (Bio Whittaker lot # 7L3790, sensitivity 0.125EU/mL) was reconstituted with 0.25 mL of test sample. The quadruplicate test samples, artificially positive test samples, negative controls and positive controls were incubated at 37°C for 60 minutes. Positive results were typified by the formation of a viscous gel that was unaffected by 180° inversion. The single preparation gave a value of less than 5 EU/mL. This assay can (and will) be repeated on the patient dose immediately prior to administration.

Table 11: Analytical Results of Radiolabeled ^{111}In-DOTA-deJ591

Test	Result
Radiolabeling yield	85%
Radiochemical Purity	>99%
Immunoreactivity	72%
Endotoxin	<5 Eu/mL

Sterility	Sterile

Lot # of deJ591: BIOV983.2-2

Large-Scale Manufacture/Process

[00306] The large-scale manufacture of the DOTA conjugated deJ591 antibody is described in the following paragraphs. The major differences from the above methodology were the use of a stirred cell, instead of a microsep centrifugal concentrator to concentrate and diafilter the antibody and the use of a Sephadex G-25 column to remove the unreacted DOTA and other reagents from the DOTA conjugated antibody. These changes were necessitated by the increase in scale. The ratios of the starting materials are given in Table 12 for a nominal 1000 milligram scale. The process may be scaled up using equivalent ratios of starting materials.

Table 12: Unit Ratios of Starting Materials

Starting Material	Unit Ratio
deJ591 antibody	X mgs
1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA)	1.25 X mgs
N-hydroxysuccinimide (NHS)	0.275 X mgs
1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)	0.3 X mgs

[00307] Aseptic practices were observed in order to minimize contamination and environmental monitoring was conducted at periodic intervals during the manufacture. All solutions, buffers and reagents used in the conjugation and purification of DOTA-deJ591 antibody were made with Water For Injection (WFI). Throughout the process, metal free components were used in the manufacture in order to avoid the chelation of any free metal residues by the DOTA moiety. In the specific case of ammonium acetate buffer and sodium phosphate buffer, the solutions were purified with Chelex 100 to remove any metal ions. Sterile, pyrogen free and metal free containers were used to mix reactants. The final bulk sterile filtration was conducted in an area that meets Class 100 specifications.

[00308] The deJ591 was prepared by buffer exchanging the antibody into metal free, 0.1 M Sodium Phosphate, pH 7.1, over a Chelex 100 (BioRad or equivalent) column. The antibody was then concentrated to approximately 10 mg/mL using a Stirred Cell Unit (Millipore or

equivalent) equipped with a 30kD cut-off membrane. The concentrated antibody was then sterile-filtered through a 0.22 μm filter.

[00309] To conjugate one gram of antibody, the active ester of DOTA was prepared by adding 6.3 mL of 0.49 M DOTA in metal free, Sodium Phosphate Buffer, pH 7.1, to 2.7 mL of 0.87 M N-hydroxysuccinimide in metal free, Sodium Phosphate Buffer, pH 7.1. To this mixture, 0.1 N Sodium Hydroxide was added until the DOTA was completely dissolved (approximately a 1:1 ratio of 0.1 M Sodium Hydroxide to DOTA/NHS solution). The pH was between 6.9 and 7.2. The solution was cooled for not less than 30 minutes at 2-8°C. To the DOTA/NHS solution, 1.5 mL of 1.0 M of EDC in Sodium Phosphate Buffer, pH 7.1, was added and allowed to cool at 2-8°C for not less than 1 hour.

[00310] The active DOTA ester was added to 1 gram of antibody and incubated overnight (12-14 hrs) at 2-8°C. The DOTA conjugated antibody was purified over a Sephadex G-25 column (Pharmacia or equivalent) in metal free, 0.3 M Ammonium Acetate Buffer, pH 7.2. The eluate fraction containing the DOTA conjugated antibody was concentrated using a Stirred Cell equipped with a 30 kD cut-off membrane to approximately 10 mg/mL. The DOTA conjugated deJ591 Antibody was then diafiltered in 0.3 M Ammonium Acetate, pH 7.2 to remove any excess reagents and diluted to a final concentration of 8.0 mg/mL prior to sterile filtering through a 0.22 μm filter.

[00311] DOTA conjugated deJ591 was tested for concentration, immunoreactivity, conjugation, endotoxin, and sterility. The endotoxin limit is based on the low clinical dose of the radiolabeled DOTA conjugated deJ591 antibody required, which ranges from 1 to 5 mg. Bioburden testing was performed on the bulk purified DOTA conjugated antibody instead of sterility because of the small batch sizes. Sterility (21 CFR 610) will be performed on the final vialed drug product. The target for immunoreactivity and number of DOTA moles per antibody was based on previous clinical experience. DOTA conjugated antibody with immunoreactivity values of as low as 72% have been successfully used in the clinic. The number of DOTA moles per antibody is based on the results from previous clinical lots.

Protein Concentration

[00312] A sample of DOTA-deJ591 was analyzed by optical density in a spectrophotometer at a wavelength of 280 nm. The extinction coefficient used for these

calculations was A_{280}, $E_{1\,cm}^{0.1\%} = 1.4$. The test sample was suitably diluted to give an absorbance reading in the working range of the assay (0.2 OD units to 1.2 OD units, linear, CV less than 2%). The acceptable limit for protein concentration is 8.0 mg/mL ± 0.5 mg/mL.

Endotoxin

[00313] Samples of DOTA-deJ591 were tested for pyrogens using a validated Limulus Amebocyte Lysate test (LAL) Gel Clot Assay (BioWhittaker or equivalent). A 0.06 EU/mL sensitivity Lysate was utilized and samples were diluted either 1:10 or 1:25 in Endotoxin free water for analysis in order to overcome the inhibition level of certain chemicals to the gel clot assay. Duplicate determinations were made for each buffer or intermediate sample during processing and the sample values needed to be equal to or less than the value obtained at the dilution level set for that buffer. A positive and negative control, as well as an inhibition control, was run with every sample. The proposed acceptable limits were not more than 5 EU per mg of DOTA-deJ591.

Bioburden

[00314] Aliquots of DOTA-deJ591 were directly inoculated in fluid thioglycollate and soybean-casein broth. The media were examined after fourteen days of incubation. As necessary, both media showed no growth after fourteen days.

Immunoreactivity

[00315] The immunoreactivity of the DOTA-deJ591 preparations was assessed according to the method of Lindmo (Lindmo T. et al. (1994) *J. Immunol. Methods* 72:77-89) which extrapolates the binding of the radiolabeled antibody at an infinite amount of excess antigen. Briefly five test solutions were prepared (in duplicate) containing 10,000 cpm of ^{111}Indium labeled-DOTA-deJ591 and various amounts of LNCaP cells or cell membranes, in a total test volume of 250 µL of 0.2 % BSA 10 mM HEPES. The solutions were incubated at 4°C for 60 minutes prior to being isolated (by centrifugation) and washed with ice cold PBS. The membranes were then counted in a gamma counter with standards representing the total radioactivity added. The data was plotted using the Lindmo method as the reciprocal of the substrate concentration (x-axis) against the reciprocal of the fraction bound (y-axis). The data

was then fitted according to a least squares linear regression method (Sigma Plot) and the y intercept used as the reciprocal of the immunoreactivity. The target for immunoreactivity was not less than 75%.

Number of DOTA Moles per antibody

[00316] The number of DOTA bound per antibody was determined using a saturation binding method with natural occurring isotope of Indium and ^{111}Indium. Multiple aliquots (minimum two) of DOTA-deJ591 were mixed with various amounts, ranging from 10 to 30 μL, of a 3.0 mM solution of $InCl_3$ (0.01 M HCl) spiked with a tracer amount of ^{111}In. The mixture was incubated at 37°C for 16 hours and then analyzed by ITLC, using silica gel impregnated glass fiber 10 cm strip (ITLC-SG, Gelman, or equivalent) and an eluant of 1% DTPA (pH 6.0). The antibody bound activity remains at the origin and free ^{111}In moves with the solvent front as an ^{111}In-DTPA complex. The relative amounts of ^{111}In and ^{111}In -DOTA-J591 was determined by cutting the ITLC strip at a R_f of 0.5 and counting the two halves with a Na(Tl)I detector. The number of binding sites was calculated by considering the molar reaction ratio between ^{111}In and DOTA-deJ591 and the observed ratio of ^{111}In and ^{111}In-DOTA-J591 detected. The target number of DOTA molecules per antibody was between 4 and 6.

[00317] The analytical results for a sample lot of DOTA conjugated deJ591 antibody are shown below in Table 13.

Table 13

Test	Proposed Acceptable Limits	Results
Appearance	Clear Colorless Solution	Clear Colorless Solution
Concentration	8.0 mg/mL ± 0.5 mg/mL	8.4 mg/mL
Endotoxin	NMT 5 EU per mg	<1.2 EU/mg
Bioburden	No growth	No growth
Immunoreactivity	For Information Only (Target NLT 75%)	95%
Number of DOTA moles per Antibody	For Information Only (Target 4-6 DOTA per Antibody)	6

[00318] The DOTA conjugation numbers for a previous lot of DOTA conjugated antibody (Biov983.2-2) and current Lot 243101 are shown in Table 14. The average number of DOTA moles per antibody for Lot Biov983.2-2 was 5.06 and for Lot 243101 was 5.96. Although the

number of moles of DOTA conjugated per antibody was slightly higher for Lot 243101, the immunoreactivity was not affected as shown in Table 15. In fact, the immunoreactivity for Lot 243101 was higher than that for the comparison lot, which is beneficial. It should be noted that other small-scale clinical lots have had immunoreactivity values of greater than 90% (data not shown).

Table 14: Comparison of the Mean Number of DOTA Molecules Conjugated to deJ591 antibody

Lot number	Known ^{111}In/DOTA-deJ591	Observed ^{111}In/DOTA-deJ591	Mean number of DOTA mols per mAb
BIOV983.2-2	Reaction ratio	TLC ratio	
A	11.76	1.338	5.03
B	17.64	2.469	5.09
Ave			5.06
Lot 243101	Reaction ratio	TLC ratio	
A	10.98	0.8608	5.90
B	16.46	1.7301	6.03
C	21.95	2.8226	5.74
D	32.93	4.3498	6.15
Ave			5.96

A= 10 μL of In-natural/^{111}In solution, B= 15 μL of In-natural/^{111}In solution, C=20 μL of In-natural/^{111}In solution, D=30 μL of In-natural/^{111}In solution

Table 15: Comparison of Immunoreactivity of DOTA-deJ591

Test	Lot BIOV983.2-2	Lot 243101
Immunoreactivity	72%	95%

Alternatives

[00319] An alternative synthesis of the DOTA-J591 immunoconjugate is as follows: 956.5 mg of deJ591 was diafiltered six times. The antibody was concentrated in a 30 kDa microsep centrifugal concentrator (Pall Filtron, MA) to approximately 15 mg/mL and diluted 12.5 fold with metal free 0.1 M Sodium phosphate at pH 7.1. This procedure was performed six times. An active ester of DOTA was created by mixing 598 mg of DOTA (1.48 mmoles) in 5.95 mL 0.1 M metal free phosphate buffer and 132 mg N-hydroxysuccinimide (1.15 mmoles) in 2.7 ml of 0.1 M metal free phosphate buffer. The pH was adjusted to 6.9-7.2 with NaOH, prior to the addition of 144 mg (0.75 mmoles) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in 1.45

mL 0.1 M metal free phosphate buffer. This reaction mixture was filtered through a 0.2 micron sterile filter and cooled on ice for 1 hour before being added to the deJ591 solution and incubated overnight at 2-8°C for 14-18 hours. The resultant DOTA-deJ591 was separated from the excess DOTA and other reactants by purifying it through a G-25 column equilibrated in 0.3 M metal free ammonium acetate. The purified conjugate was concentrated to 10 mg/mL in a stirred cell unit and washed with 0.3 M ammonium acetate, then sterilized by filtration through a 0.22 um filter and stored in a sterile polypropylene vial at 2-8°C.

[00320] Yet another alternative is to use a conjugation process that involves pure DOTA-NHS mono-active ester (commercially available from Macrocyclics) so as to achieve better control over the amount and purity of the DOTA-NHS mono-active ester used in the conjugation process, as well as to limit unanticipated chemical side-reactions produced when DOTA is activated in-situ. Problems associated with the use of in-situ acitivated DOTA (i.e., DOTA activated without any purification prior to being added to the antibody) include: fluctuation of DOTA/antibody incorporation ratio due to the variable generation of DOTA-NHS; the use of a large excess of DOTA which needs to be purified away from the conjugated antibody and can compete for binding to radioactive isotopes; unreacted EDAC can result in the cross-linking of lysine and glutamic or aspartic acid residues on proteins and consequently the formation of undersired protein aggregates; and there is the possibility that two or more active esters are formed on a significant fraction of the activated DOTA molecules which enables proteins to become crosslinked and uses additional carboxylate groups that are needed for metal coordination.

[00321] To minimize the loss of antibody binding activity resulting from its conjugation to DOTA, it is desirable to incorporate about 2-10, preferably about 5-7 DOTA molecules per antibody molecule. Using DOTA-NHS monoactivated ester, a 3-30 fold excess of DOTA-NHS relative to antibody produces this desired level of DOTA incorporation. Studies are currently being conducted using input ratios of DOTA-NHS to antibody of 7:1, 9:1, 11:1, 15:1, 20:1, and 30:1.

[00322] The protocol is as follows. Starting materials: J591 antibody, 10.5 mg/ml is sodium phosphate buffer (0.1M, pH 7.1), treated with Chelex 100 resin (1 ml resin per 10 mg of antibody); 0.3 M ammonium acetate buffer (pH 7.0), treated with Chelex 100 resin (20 ml resin per one liter buffer); DOTA-NHS.PF6 (FW 646.4), Macrocyclics, Dallas, TX. Experimental

procedures: Three polypropylene vials were separately charged with 2.0 ml of J591 antibody (10.5 mg/ml, 143 nmol) and chilled on ice over a period of 30 minutes. 3.3 mg of DOTA-NHS were dissolved in 0.356 ml of metal-free water (treated with Chelex 100 resin and pre-chilled on ice for 30 minutes) to give a concentration of 14.3 nmol per microliter. To the three antibody solutions were separately added 70, 90, and 110 microliters of this freshly prepared DOTA-NHS solution. The reaction mixtures were slowly stirred with magnetic stirring bars at room temperature over a period of 4 hours, and then diluted with 0.3 M ammonium acetate buffer (pH 7.0, Chelex 100 treated) to 15 ml in CentriCon-30 for buffer exchange. The concentrates (about 1.5 ml) were then diluted again to 15 ml and concentrated down to 1.5 ml. These concentrates were then filtered through 0.2 micron filters. The CentriCon tube and filters were washed with a small amount of ammonium acetate buffer and filtered into final products (total of 2.0 ml each).

[00323] Antibodies conjugated to DOTA using the 7:1, 9:1, and 11:1 input ration of DOTA-NHS to antibody have been analyzed for ^{90}Y binding stability and the formation of protein aggregates. All three conjugates displayed a high percentage of stability after 2-3 days of labeling with ^{111}In or ^{90}Y, in the presence of PBS, DTPA chelate challenger, serum and transferrin. In addition, all three conjugates displayed little or no process-related aggregate formation. The remaining three conjugates, produced using the higher DOTA-NHS to antibody input ratios, are currently being analyzed.

[00324] The conjugation of DOTA to antibodies is not limited to NHS acitivated DOTA. The DOTA-HOBT active ester can be used in place of DOTA-NHS, as well as other activation methods known in the art, such as the use of a mixed anhydride of ethyl chloroformate or isobutyl chloroformate, p-nitrophenyl ester.

Example 2- Pharmacokinetics and Biodistribution of ^{131}I- and ^{111}In-labeled deJ591 and murine J415 in Nude Mice Bearing LNCaP Human Prostate Tumors

[00325] In nude mice bearing PSMA-positive human LNCaP tumors, the pharmacokinetics, biodistibution and tumor uptake of monoclonal deJ591 and murine J415 antibodies radiolabeled with ^{131}I or ^{111}In was analyzed. Autoradiographic studies were performed to identify intra-tumoral distribution of radiolabeled MAbs.

[00326] De J591 and J415 were labeled with ^{131}I using the iodogen method (see Franker and Speck (1978), *Biochem Biophys Res Commun* 80:849-57) to a specific activity of

400MBq/mg (21, 23). For ^{111}In labeling, the J415 and deJ591 antibodies were first conjugated with 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) and then labeled with ^{111}In to produce specific activities of 200 MBq/mg.

[00327] Prostate carcinoma cell lines LNCaP, DU145 and PC3 (American Type Culture Collection, Rockville, MD) were grown in RPMI 1640, supplemented with 10 % fetal calf serum, at a temperature of 37°C in an environment containing 5% CO_2. Prior to use, the cells were trypsinized, counted and suspended in Matrigel (Collaborative Biomedical Products, Bedford, MA). Nu/Nu Balb C mice 8-10 weeks of age were inoculated, in the right and left flanks, with a suspension of 5 x 10^6 LNCaP cells in Matrigel (BD Biosciences, Bedford MA). After a period of 14-18 days, tumors (100-300 mg) had developed. The PSMA-negative DU145 and PC3 cells were implanted in nude mice in an identical manner.

[00328] The PSMA-positive and PSMA-negative tumor bearing mice were injected, via the tail vein, with 80 KBq of the iodinated MAb (400 MBq/mg) in 200 µL of PBS (pH 7.4, 0.2% BSA). Groups of animals (3-8/group) were sacrificed after 2, 4 or 6 days. The major organs and tumors were recovered. The tissue samples were weighed and counted, with appropriate standards in an automatic NaI(Tl) counter. These measured relative activity data (cpm) were background corrected and expressed as a percentage of the injected dose per gram (%ID/g). These data were also fitted with a least squares regression analysis (Microcal Origin, Northampton, MA) to determine the biological clearance of the various agents. J415, J591 and 7E11 were labeled with ^{111}In (100MBq/mg). 80KBq of the ^{111}In labeled MAbs were injected in groups of animals. The %ID/g in various organs and tumor tissues was determined at 2-6 days post injection in a similar way as described for ^{131}I labeled MAbs.

[00329] For the imaging studies, animals were injected with 2 MBq of ^{111}In-DOTA-J591. On days 1, 2, 3, 4 and 6 post-injection, the mice were sedated with ketamine/xylazine 100 mg/kg/10 mg/kg IP. The mice were imaged with a gamma camera (Transcam, ADAC Laboratories, Milpitas, CA) equipped with a pinhole collimator. The images were acquired in a 256 x 256 matrix for 1000 seconds using a 20% window of 245 KeV photopeak of ^{111}In.

[00330] For several animals (n=20), harvested tumor samples were immediately cooled in liquid nitrogen and frozen in embedding medium (O.C.T. 4583, Sakura Finetec, Torrance CA). Twenty micron sections were cut and the tumor sections were either fixed with acetone and placed in direct contact with a sheet of photographic film (Biomax, Kodak, Rochester, NY) or

stained with hematoxin/eosin prior to exposure of the film. Tumors from control animals were collected and cut into 10 μm sections. These sections were soaked in tris buffer (170 mM, pH 7.4, with 2 mM $CaCl_2$ and 5 mM KCl) for 15 minutes, washed with Tris buffer (170 mM, pH 7.4) and incubated with ^{131}I-J591 MAb for 1 hour at 4°C. Non-specific binding was determined in the presence of 100 nM J591 MAb. These sections were then washed 3 times with PBS (containing 0.2% BSA) and once with Tris buffer prior to being fixed with acetone and then exposed to photographic film.

Biodistribution of ^{131}I labeled MAbs

[00331] In nude mice bearing LNCaP tumors, the biodistibution and tumor uptake of ^{131}I labeled deJ591 and J415 was compared to that of ^{131}I-7E11. At 2 days post injection deJ591, J415, and 7E11 had similar tumor uptake and blood pool activity. On day 6, the tumor uptake of J415 (15.4±1.1) was significantly higher compared to that of deJ591 (9.58±1.1). The blood activity of both deJ591 and J415 was significantly lower compared to that of 7E11. With the ^{131}I labeled MAbs, the tumor/blood and tumor/muscle ratios were significantly higher with J415 than with J591 or with 7E11.

[00332] In order to assess the specificity of radiolabeled MAb localization in PSMA-positive LNCaP tumors, the uptake of ^{131}I labeled deJ591 and J415 in selected organs was compared to that of an irrelevant IgG antibody. At one day post injection, the tumor uptake (%injected dose/gram) of both J415 (12.2±3.24) and deJ591 (8.55±1.29) was significantly higher compared to that of an irrelevant antibody (4.41±0.40). The uptake in lungs, kidney, muscle was similar with all three antibodies. In a second control study, the tumor uptake of ^{131}I-deJ591 was determined in nude mice bearing the PSMA-negative prostate tumors (PC3 and DU145). At 4 days post injection, the tumor uptake of ^{131}I-deJ591 was only 0.66 ±0.07 % ID/g in PC3 tumors (n=10) and 0.55 ±0.03 %ID/g in DU145 tumors (n=6). In contrast, the tumor uptake of ^{131}I-J591 was 11.4 ±1.49 % ID/g in PSMA-positive LNCaP tumors, significantly greater than in PSMA-negative tumors ($p<0.01$).

Biodistribution of ^{111}In labeled MAbs

[00333] With ^{111}In, the tumor uptake of deJ591 and J415 gradually increased with time and is quite similar to that of 7E11. The kinetics of blood clearance for both J415 and J591 is

faster compared to 7E11. At 6 days post-injection, the blood activity of J415 (2.63±0.23) and deJ591 (2.52±0.16) is about 50% that of 7E11 (4.16±0.21). As a consequence, the tumor/blood ratios with J415 and deJ591 are significantly higher compared to that of 7E11. There were minor differences in the uptake of these three antibodies in liver, spleen and kidney.

[00334] The serial gamma camera images of a nude mouse clearly show the intense tumor accumulation of ^{111}In-DOTA-deJ591. On day 1, the single tumor (ca 250 mg) on the right hind quarter, the blood pool and the liver are well visualized. But in the later images, while the activity has cleared from the blood pool, the tumor accumulation become gradually more intense compared to that of liver activity.

Autoradiography

[00335] Tumor specimens (n = 20) were harvested for hematoxylin (H) and eosin (E) staining and autoradiography to study the intra-tumoral biodistribution of ^{131}I-labeled MAbs 4 to 6 days after intravenous injection. The H and E stains reveal a considerable amount of necrosis, averaging 50% of the cross-sectional area, in all specimens studied. The autoradiographs reveal a focal, somewhat heterogeneous, distribution pattern with all three antibodies. Interestingly, the biodistribution pattern with MAbs to $PSMA_{int}$ and $PSMA_{ext}$ reveal almost reciprocal patterns. That is, 7E11 (anti-$PSMA_{int}$) distinctly favors localization to areas of necrosis whereas J415 and J591 (anti-$PSMA_{ext}$) demonstrate a distinct preferential accumulation in areas of viable tumor. Ex vivo autoradiography, where ^{131}I-J591 was incubated directly on the tissue section, demonstrated a homogeneous binding pattern.

Conclusions

[00336] The localization of radiolabeled J591 and J415 in PSMA positive LNCaP tumors is highly specific. These results clearly demonstrate that PSMA-specific internalizing antibodies such as deJ591 and deJ415 may be the ideal MAbs for the development of novel therapeutic methods to target the delivery of beta emitting radionuclides (^{131}I, ^{90}Y, ^{177}Lu) for the treatment of PSMA-positive tumors.

[00337] MAbs to $PSMA_{ext}$ had a reciprocal pattern to 7E11, with localization concentrated in areas of viable tumor. The inability of 7E11 to target well vascularized, viable tumor sites probably explains the inability of ProstaScint® to image bone metastases as well as to explain its

failure in RIT trials. By targeting viable tumors, mAbs to $PSMA_{ext}$, like deJ591 and deJ415, will have a better therapeutic effect. In addition, their ability to target viable tumor imparts better ability to localize well-vascularized sites in the bone marrow.

Example 3 - Animal Studies Using ^{90}Y-DOTA-deJ591

[00338] In *in vitro* and *in vivo* animal models, ^{90}Y-DOTA-deJ591 has demonstrated substantial anti-tumor activity. In these studies, immunodeficient 'nude' mice were implanted intramuscularly with PSMA-expressing human prostate cancer cells (LNCaP). In some studies, the same animals were simultaneously implanted in the opposite thigh with a PSMA-absent human prostate cancer line (PC3). Cancers were allowed to 'establish' for a period of approximately 2 weeks during which time the cancer develops a blood supply allowing further growth. At the time of treatment initiation, the cancer implants average 1.0 cm in diameter (or approximately 5% of the animal's body weight).

[00339] Four groups of mice received a single injection of 1.3, 3.7, 5.55 or 7.4 MBq of ^{90}Y-DOTA-deJ591. At a 1.3 MBq dose level, there was a mixed response with minimal reduction in tumor growth rate. However, at doses between 3.7-7.4 MBq, a clear anti-tumor dose-response relationship was observed. There was a 30, 55 and 90% reduction in mean tumor volume and a progressive dose-related delay in tumor re-growth of 10, 35 and 60 days at 3.7, 5.55, and 7.4 MBq dose levels, respectively. More than 70% of mice that received 3.7-5.55 MBq lived significantly longer than the controls and the mean survival time (MST) increased to 80-100 days compared to 40 days for controls.

[00340] Three groups of mice received 1.11, 2.22 or 3.33 MBq of ^{90}Y-DOTA-huJ591 every 28 days for 3 doses. At the 1.11 MBq dose level, there was minimal tumor growth during a period of 75-80 days. At 2.22 and 3.33 MBq dose levels, there was a 50-70% reduction in the mean tumor size at day 60 followed by gradual increase in tumor size thereafter. The MST increased by about 200% at 1.11 and 2.22 MBq dose levels compared to control group (120 vs. 40 days).

[00341] These studies confirm significant improvement in survival of the ^{90}Y-DOTA-deJ591 treated animals. The PSMA-absent cancers do not respond to treatment demonstrating the specificity of the treatment.

Example 4 - Animal Studies Using ^{177}Lu–DOTA-deJ591

[00342] Nude mice bearing LNCaP tumors treated with ^{177}Lu–DOTA-deJ591 exhibited a response similar to the ^{90}Y-DOTA-deJ591 treated animals. In this study mice with LNCaP tumors (300-400 mg) were divided into three groups. A control group received no treatment. Group-2 received 200μCi and Group-3 received 300μCi of ^{177}Lu-DOTA-deJ591. ^{177}Lu-DOTA-deJ591 was, in a dose dependent manner, able to reduce the mean tumor mass by 80-97% at the two dose levels. The control group showed a progressive increase in tumor size, which was accompanied by a steady loss of body weight and the mice were sacrificed by fifty-three days because of low body mass. The animals treated with 200 μCi ^{177}Lu-DOTA-deJ591 had tumor shrinkage up to twenty days post injection, and thereafter tumor regrowth was seen in some animals. The same group also had a nadir in body mass of about 90% (of baseline wt) at twenty days post injection, but thereafter a steady rise in the mean body weight to 100-105% of the starting mass. At ninety days, four out of eleven mice had no palpable tumors. The mice treated with 300 μCi of ^{177}Lu-DOTA-deJ591 had tumor shrinkage up to forty days post injection, and thereafter tumor regrowth was seen in some animals. The same group also had a nadir in body mass of about 90% at twenty days, but thereafter a steady rise in the mean body weight to 105-110 % of the starting mass. At ninety days, five out of eleven mice had no palpable tumors.

Example 5 - Human Trial with ^{131}I - J591 (murine); Phase I Clinical Trial Targeting A Monoclonal Antibody (mAb) To The Extracellular Domain Of Prostate Specific Membrane Antigen ($Psma_{ext}$) In Hormone-Independent Patients

[00343] Hormone-independent patients with rising PSA levels and acceptable hematologic, hepatic and renal function received a single dose of murine mAb J591. Doses are escalated (from 0.5-300 mg) in groups of three to six patients. Each dose included $\leq$ 1.0 mg J591 labeled with 10 mCi ^{131}I iodine as a tracer plus "cold" mAb. The dose levels used ranged from 0.5 to 300.0 mg as follows: 0.5 mg, 1.0 mg, 2.0 mg, 5.0 mg, 10 mg, 25 mg, 50 mg, 100 mg, 125 mg, 150 mg, 200 mg, 250 mg, and 300 mg. Blood and urine were collected to monitor pharmacokinetics, toxicity and human anti-mouse antibody (HAMA) response. Patients were imaged on the day of injection (day 0), as well as days 2, 4 and 6 to track mAb targeting.

[00344] Thirty-three patients with hormone-independent prostate cancer were entered into the phase I biodistribution trial of trace-labeled ^{131}I- J591. These patients received doses ranging

from 0.5 to 300.0 mg, in each case conjugated with 10mCi ^{131}I. In approximately 80% of patients where disease sites had been imaged by conventional studies (CT/MRI and/or bone scan), known sites of prostate cancer metastases can be imaged. Both soft tissue and bony sites could be visualized on mAb scan. Targeting was specific for prostate cancer sites without apparent localization to non-cancer sites. The mean effective serum residence time of ^{131}I-mJ591 was determined to be 44.0 hours (median 47.4 hrs). Eight out of sixteen evaluable patients developed a human anti-murine antibody (HAMA) response. That is, their immune systems recognized the foreign nature of the mouse-derived antibody. Only one patient had an adverse event related to the murine antibody. This patient had a severe allergic (anaphylactic) reaction to the murine mAb later determined to be due to prior (unknown) exposure to murine antibody used in purification of another experimental drug with which the patient had previously been treated. In sum, thirty-three patients were evaluated for targeting: twenty-seven out of thirty-three patients had positive bone scans, with twenty-four out of twenty-seven (about 90%) having positive monoclonal antibody scans; nine out of thirty-three patients had positive soft tissue (CT scans), with eight out of these nine (about 90%) having positive monoclonal antibody scans.

[00345] Development of a HAMA response, which occurs in most immunocompetent patients who receive murine antibodies, precludes repeated treatments with a foreign species-derived antibody. In order to allow for multiple treatments, murine antibody molecules can be "de-immunized" using molecular engineering techniques which remove foreign (mouse) amino acid sequences and replace them genetically with known homologous human sequences. As indicated above, murineJ591 has undergone de-immunization resulting in "deJ591".

[00346] In conclusion, mAb to $PSMA_{ext}$ targets *in vivo* specifically to disseminated prostate cancer sites in both bone and soft tissue with no significant 'adverse' localization and no significant toxicity.

Example 6 - Single Human Patient Study Using ^{90}Y-DOTA-deJ591

[00347] A single patient was treated with mAb deJ591. This patient had bulky, poorly differentiated prostate cancer and had failed multiple courses of external beam radiotherapy as well as multiple forms of hormonal and non-hormonal chemotherapy. The patient was treated under a single patient IND and received a total of twelve doses of deJ591 over a course of five months, ranging from 10 mg to 200 mg. Four doses (#1, 3, 6 and 11) were trace-radiolabeled

with either ^{131}Iodine or ^{111}Indium for pharmacokinetic determinations and biodistribution. The mean effective serum residence time of ^{131}I-deJ591 ranged from 31.9 to 51.3 hours, depending on the dose. Tumor localization to known tumor sites was excellent after each of the radiolabeled doses over the five month period. Dose twelve was radiolabeled with a therapeutic quantity of ^{90}Yttrium (19mCi) calculated to deliver less than a 150 rad dose to the blood. This dose was determined in consultation with the FDA and took into account prior radiotherapy delivered by external beam and by ^{131}Iodine. No detectable human anti-deimmunized antibody (immune) response developed in this patient. The patient's platelet count dropped to 64,000 (normal: 150,000-300,000) at five weeks post ^{90}Y-DOTA-deJ591 administration (as an anticipated result of radiation to the bone marrow) prior to spontaneously returning to normal levels. No other hematologic or non-hematologic toxicity occurred. The patient experienced no side effects. The patient's PSA declined from 63 at the time of deJ591-DOTA-^{90}Y administration to 36. No measurable reduction in tumor number or size occurred. This patient succumbed to his metastatic prostate cancer ten and one-half months after initiating treatment with ^{90}Y-DOTA-deJ591.

Example 7 - Human Trial with ^{111}In-DOTA-deJ591 - Phase I Trial of ^{111}Indium labeled deimmunized Monoclonal Antibody (mAb) deJ591 to Prostate Specific Membrane Antigen/Extracellular Domain (PSMAext)

[00348] This example describes the results of a clinical trial of deJ591 to assess mAb targeting, toxicity, pharmacokinetics (PK) and immunogenicity (human anti-deimmunized Ab) of this genetically engineered mAb. DeJ591 is a strong mediator of antibody dependent cellular cytotoxicity (ADCC). As PSMA is expressed in tumor, but not normal, vascular endothelium of all cancers, the diagnostic and therapeutic utility of deJ591 may extend beyond prostate cancer to other cancers.

[00349] Patients with recurrent, progressing prostate cancer received four weekly doses of ^{111}In-DOTA-deJ591. Doses were escalated in cohorts of three patients and ranged from 62.5-500 mg/m2 (total). Dose level is shown in Table 16. Each dose included 0.02-1.0 mg deJ591 trace-labeled with 0.1-5 mCi ^{111}In via a mAb-DOTA chelate, with the remainder of the dose consisting of unlabeled deJ591. After the first dose, patients were imaged on the day of injection (day 0) and days 1, 2, 4 and 7.

Table 16: ^{111}In-DOTA-deJ591 Dosage

Dose Level	Loading Dose (mg/m^2)	Maintenance Dose (mg/m^2)	Total Dose (mg/m^2)
1	25	12.5	62.5
2	50	25	125
3	100	50	250
4	200	100	500

1-2mg ^{111}In-DOTA-deJ591 (0.2-10 mCi), with the balance of the dose as "cold" deJ591.

[00350] Serum PK, immune reaction and toxicity were evaluated after each dose for a minimum of 12 weeks.

[00351] Fifteen patients were initially entered in the trial. Thirteen patients received all four planned doses; two patients received ≤ 1 dose. One patient became hypotensive 5 minutes into his first infusion due to a rapid infusion rate. The second patient who did not complete treatment was withdrawn from the study after one week due to rapid disease progression rendering him no longer eligible. Neither this latter patient nor the remaining thirteen patients experienced any toxicity or side effects. Ten out of the thirteen patients had positive bone scans; all ten demonstrated excellent mAb targeting to bony sites. Three patients had soft tissue disease as measured by a CT scan; two demonstrated mAb targeting to these sites, while the third patient that was ^{111}In-DOTA-deJ591 negative had a radiated pelvic mass that had not changed size in >18 months. No mAb targeting to non-prostate cancer sites was noted. None of the patients developed an immune reaction to the antibod. Plasma half-life of deJ591 varied with dose.

[00352] In conclusion, deJ591 is non-immunogenic and targets sensitively and specifically to both bone and soft tissue.

Example 8 - Human Trial with ^{111}In-DOTA-deJ591; Targeting of Hormone Refractory Prostate Cancer

[00353] This example describes the results of a clinical trial of deJ591 to assess mAb targeting, biodistribution, and pharmacokinetics and to optimize antibody dose for radioimmunotherapy with this mAb in patients exhibiting hormone refractory prostate cancer.

[00354] Twenty-six patients exhibiting hormone refractory prostate cancer were injected with a single dose of ^{111}In-DOTA-deJ591, consisting of 20mg deJ591 labeled with 185 MBq of ^{111}In-DOTA. All patients underwent whole body and SPECT imaging on days 0 (the day of injection), 3, and 6 of ^{111}In-DOTA-deJ591 injection. ^{111}In-DOTA-deJ591 imaging results were compared with CT, MRI, and bone scan. All patients had rising PSA levels on three consecutive measurements.

[00355] Twenty-two of the twenty-six patients had imagable disease on routine imaging modalities, while four patients had no imagable disease. Imaging data revealed ^{111}In-DOTA-deJ591 tumor targeting in sixteen of the twenty-two (72.7%) patients who had imagable disease. Targeting was best observed on day 3. No additional (unknown) sites were detected on SPECT imaging. Targeted metastatic sites were in the bone or bone marrow in twelve patients, in the soft tissue in two patients, and in both the bone and soft tissue in two patients.

[00356] ^{111}In-DOTA-deJ591 imaging was false negative in four of the twenty-two (18%) patients who had imagable disease. Non-targeted metastatic sites were in the soft tissue in three patients and in both the bone and soft tissue in one patient.

[00357] Accumulation of ^{111}In-DOTA-deJ591 in the prostate gland was generally minimal in patients with intact but irradiated prostate. Among physiological sites, highest uptake was observed in the liver, with 27+/-1.7% uptake at day 6. The absorbed dose by the liver was 2.8+/-0.25 rads/mCi with ^{111}In and 20.1+/-2.1 rads/mCi with ^{90}Y. Plasma clearance (T1/2) of ^{111}In-DOTA-deJ591 was 34+/-5 hours.

[00358] ^{111}In-DOTA-deJ591 specifically targets hormone refractory prostate tumors and is an effective vehicle to target hormone refractory advanced prostate cancer with radioactivity or cytotoxins.

Example 9 - Targeting of Hormone Refractory Prostate Cancer with ^{111}In-DOTA-deJ591 or ^{177}Lu-DOTA-deJ591

[00359] Imaging studies with ^{177}Lu-DOTA-deJ591, like those described in Example 8 for ^{111}In-DOTA-deJ591, have produced similar results. The following summary represents a combination of the results obtained for ^{177}Lu and ^{111}In.

[00360] Thirty-nine patients with hormone-refractory prostate cancer have thus far been enrolled in one of several clinical trials utilizing deJ591. Nuclear imaging with either ^{111}In or

^{177}Lu labeled deJ591 was performed as an initial step of each trial. ^{111}In, which is a γ-emitter, was used either as tracer for "naked" mAb or as a surrogate imaging agent prior to the administration of ^{90}Y (a pure β emitter which does not image on radionuclide scans). ^{177}Lu emits both γ and β particles, allowing direct imaging, and can be used as both an imaging and a therapeutic agent. The antibody scans were compared to standard imaging studies (bone, CT or MR scans) obtained on each patient to assess the underlying sensitivity, specificity, and accuracy of deJ591 targeting to metastatic sites. Bony and soft tissue metastases were evaluated independently.

[00361] Results have been obtained for bony metastasis in twenty-three patients, and soft-tissue metastasis in twenty-five patients. Fifteen patients had bone metastases on bone scan, which were accurately targeted by deJ591 in twelve patients (80%). In these twelve patients, every cancerous lesion seen on bone scan was identified on the deJ591 scan. All seven patients with negative bone scans were negative on the deJ591 scan (100% specificity). For soft tissue metastases, eight out of nine patients with soft tissue masses on conventional imaging demonstrated accurate targeting using deJ591 (89%). The one false negative patient had retroperitoneal adenopathy measuring 8mm not seen on mAb scan, but whose bony lesions were all targeted. Fifteen patients without documented soft tissue metastases had negative mAb scans (100%). One patient without soft tissue metastases on initial standard imaging but lesions identified on mAb scan subsequently proved positive on standard imaging. Overall, there was a 91% concordance between standard imaging and mAb scans for soft tissue and bony metastases.

[00362] DeJ591 accurately targets known bony or soft tissue metastases in the vast majority of patients. Additionally, one previously unseen metastatic site was demonstrated on mAb scan and later confirmed with CT imaging. DeJ591 is a highly sensitive and specific agent for targeting metastatic prostate cancer lesions.

Example 10 - Human Trial with ^{90}Y-DOTA-deJ591; Phase I Trial of De-immunized mAb deJ591-DOTA-^{90}Yttrium In Patients With Relapsed Prostate Cancer

[00363] A phase I trial of escalating doses of ^{90}Y-DOTA-deJ591 therapy of patients with recurrent/relapsing prostate cancer was conducted. Doses started at 5 mCi/m^2 and were escalated in increments of +2.5-5 mCi/m^2 for cohorts of three to six patients. The design of this study is summarized as follows: ^{111}In-DOTA-deJ591 was administered to patients so as to determine the

biodistribution of the antibody and the associated dosimetry; and ^{90}Y-DOTA-deJ591 was administered 7-10 days later at 5.0, 10, 15, 17.5, and 20 mCi/m^2. All administrations were by intravenous infusion at a rate of about 5mg/min. DeJ591-DOTA was labeled at a specific activity between 3-5mCi-^{90}Y/mg antibody to reach the defined dose of ^{90}Y, with the balance brought up to 20 mg total deJ591 with "cold" deJ591. Dosages were administered with 6-8 weeks between dose levels. Subsequent doses of ^{90}Y-DOTA-deJ591 were allowed.

[00364] The subjects for this trial had prostate cancer that had relapsed after definitive therapy (e.g. surgery and/or radiation) and for whom no curative standard therapy exists.

[00365] The objectives of this trial are to: (1) define the toxicity and maximum tolerated dose (MTD) of repeated (fractionated) doses of de-immunized monoclonal antibody (mAb) deJ591-DOTA-^{90}Yttrium (^{90}Y) in patients who have recurrent and/or metastatic prostate cancer; (2) define the pharmacokinetics of deJ591-DOTA-^{90}Y; (3) define the human anti-deimmunized antibody immune response to deJ591-DOTA-^{90}Y; and (4) define the preliminary efficacy (response rate) of repeated (fractionated) doses of deJ591-DOTA-^{90}Y.

Treatment Protocol:

[00366] Patients who developed ≥ grade 2 allergic reaction as a result of ^{90}Y-DOTA-deJ591 would not receive further DOTA-deJ591 and would be followed for toxicity.

[00367] Patients were followed for a minimum of 12 weeks after the deJ591-DOTA-^{90}Y administration. If the patient's disease was stable or responding at 12 weeks after his last dose, he was followed until progression.

[00368] The follow-up study consisted of gathering the information shown in Table 17, below, at the indicated times.

Table 17: Follow-up Analyses

Medical history	Day of Rx, week 1, 2, 4, 6, 8, and 12, then every 12 weeks thereafter until progression
Physical exam (focused)	Day of Rx, week 1, 2, 4, 6, 8, and 12, then every 12 weeks thereafter until progression
Performance status	Day of Rx, week 1, 2, 4, 6, 8, and 12, then every 12 weeks thereafter until progression
PSA, PAP, alkaline phosphatase	Day of Rx, week 1, 2, 4, 6, 8, and 12, then every 12 weeks thereafter until progression
Testosterone	Week 4, then every 6 months thereafter until

	progression
Human anti-deimmunized Ab	Day of Rx, week 2, 4, 8, and 12
CBC, differential, platelet count	Day of Rx, week 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, and 8, then every 4 weeks thereafter until stable, and then every 12 weeks until progression. Monitor qod if ANC <1000 and/or platelets <50,000
Electrolytes, BUN, creatinine	Day of Rx, week 1, 2, 4, and 8, then every 4 weeks until stable, and then every 12 weeks until progression
Total protein, albumin, bilirubin, GGTP, AST, ALT, LDH	Day of Rx, week 1, 2, 4, and 8, then every 4 weeks until stable, and then every 12 weeks until progression
Urinalysis	Day of Rx, week 1, 2, and 4, then every 4 weeks unless normal or baseline
CT or MRI	Week 12, then every 12 weeks (if measurable or evaluable disease present at entry and/or if patient classified as a "responder")
Bone scan	Week 12, then every 12 weeks (if evaluable disease present at entry and/or if patient classified as a "responder")
CXR	Week 12, then every 12 weeks (if disease present at entry)
Weight	Day of Rx, week 1, 2, 4, 8, and 12, then every 12 weeks until progression
Appetite	Day of Rx, week 1, 2, 4, 8, and 12, then every 12 weeks until progression
Bone pain	Day of Rx, week 1, 2, 4, 8, and 12, then every 12 weeks until progression
Analgesic intake	Day of Rx, week 1, 2, 4, 8, and 12, then every 12 weeks until progression

Pharmacokinetics:

[00369] Following injection of ^{111}In -DOTA-deJ591, blood samples were obtained at 10 min, 1, 2, 4, 24 hours and days 2, 3, 4 and 7. The percent injected dose (% I.D.) in blood was determined by measuring an aliquot of blood along with a known ^{111}In standard. Similar blood samples were taken at the same interval after the ^{90}Y- DOTA-deJ591. The % I.D. in blood was determined by measuring an aliquot of blood along with a known ^{111}In or ^{90}Y standard.

Toxicity:

[00370] NCI CTEP Common Toxicity Criteria (CTC), version 2 (April, 1999) was utilized. Since CTEP has standardized the CTC, the NCI does not require inclusion of the CTC

within this document. All treatment areas have access to a copy of the CTC version 2.0. A copy may also be downloaded from the CTEP web site.

Definition of Dose Limiting Toxicity (DLT):

[00371] Hematologic toxicity: grade 4 granulocytopenia [ANC <500/mm^2]; grade 4 thrombocytopenia [platelet count < 10,000/mm^2]; or febrile neutropenia or neutropenic infection as defined by the CTC. Other toxicity: grade ≥ 3 non-hematologic toxicity attributable to ^{90}Y-DOTA-deJ591.

Definition of Maximum Tolerated Dose (MTD):

[00372] The MTD is defined as the dose level at which 0/6 or 1/6 patients experience DLT with the next higher dose level having 2 or more patients experiencing DLT. Once the MTD has been reached, at least 6 patients should be evaluated at that dose to better determine the toxicities of the regimen and the pharmacokinetics of ^{90}Y-DOTA-deJ591.

[00373] Allergic events will be managed as follows: rash, pruritis, urticaria and wheezing will be treated with benadryl and/or steroids as clinically appropriate. Anaphylaxis or anaphylactoid signs or symptoms can be treated with steroids and/or epinephrine as clinically indicated.

Specific interventions solely for the purpose of the study:

[00374] Other than the actual administration of deJ591 and related studies to define the pharmacokinetics and biodistribution of the mAb, the other interventions (labs, imaging studies, office visits) performed are standard procedures. Some of the lab tests would not typically be done in the setting of prostate cancer (e.g., immune reaction levels). Other lab and radiographic procedures, although standard in the management of patients with prostate cancer, may be done at greater frequency than typical.

Criteria for therapeutic response:

[00375] Prostate cancer progression is manifest by rising PSA levels, new lesions on bone scan, new disease-related symptoms and, less commonly, increasing size of a measurable soft

tissue mass. Response is commonly assessed either biochemically (PSA change) or by change in size of a measurable lesion/s.

[00376] Biochemical (PSA) response can be determined by comparing the nadir PSA level after therapy to the baseline, pre-treatment PSA determined just prior to initiating therapy. A decline of >50% has been demonstrated by numerous investigators (Petrylak, D.P. et al. (1992) *Cancer* 70:2870-78; Kelly W.K., et al. (1999) *J Clin Oncol* 11:607-15; Kantoff P.W., et al. (1999) *J Clin Oncol* 17:2506-13, 1999; Smith, D.C., et al. (1998) *J Clin Oncol* 16:1835-43) to correlate with improved survival. In addition, Scher, et al (Scher H.I., et al. (1999) *JNCI* 91:244-51) have demonstrated that a PSA which either declines or shows no increase from baseline at either 8 or 12 weeks after initiating therapy correlates with improved survival compared to patients whose PSA rises despite therapy.

[00377] In patients with measurable disease: complete response is defined as complete disappearance of all measurable lesions by physical examination or imaging studies with no appearance of new lesions for $\geq$ 2 months. Partial response: is defined as a 50% or greater reduction in the sum of the products of the longest perpendicular diameters of all measurable lesions. There may be no new lesions. Stable disease: patients who do not meet the criteria of partial response and who are without signs of progressive disease for $\geq$ 2 months. Progressive disease is defined as a greater than 25% increase in the sum of the products of the longest perpendicular diameters of the indicator lesions, the appearance of new lesions or a rise in prostate specific antigen above pre-treatment baseline.

[00378] Duration of response: typically, the first sign of progression will be a rise in serum PSA. In this trial the duration of response will be the time interval from treatment initiation (^{90}Y-DOTA-deJ591) until progression is documented by either a confirmed rise in PSA, enlargement of the measurable lesion/s, or new lesion/s on imaging studies. The rising PSA must be confirmed by a second, serially rising PSA and the duration will be defined as the time from initiation of treatment to the time of the first rising PSA.

Results:

[00379] Twenty patients have been treated according to this protocol, and nineteen of the twenty can currently be evaluated. All twenty patients had failed one or more forms of hormone therapy, and eleven of the twenty patients had failed at least one chemotherapy regimen.

Furthermore, the patients all had increasing PSA levels and a minimum platelet count of 150,000.

[00380] Blood chemistry, hematology and PSA levels were monitored for twelve weeks or longer. No significant changes were observed in blood chemistry, or liver or kidney functions. Hematological changes in platelets and white blood cell levels were observed at all dose levels. Toxicity was dose-related and limited to reversible myelosuppression (primarily thrombocytopenia). Grade 3-4 thrombocytopenia was observed at 15-20 mCi/m^2. The maximum tolerated dose was estimated to be less than or equal to 20 mCi/m^2. ^{90}Y-deJ591 radiation dosimetry estimates based on ^{111}In-deJ591 imaging studies indicate that the organ dose to liver, kidney, spleen, and bone marrow are 20, 19, 18, and 1.7 rads/mCi, respectively. No patients developed an immune reaction.

[00381] Dose-related anti-tumor effects were noted. At the first two dose levels (5 and 10 mCi/m2), five out of the eleven (45%) patients had PSA values that continued to increase despite treatment, while six out of the eleven (55%) patients achieved an average 23% reduction in PSA levels. At 15 mCi/m2, one out of the four patients (25%) progressed, while three out of the four (75%) patients achieved an average 25% reduction in PSA levels. At 20 mCi/m2, all four patients achieved an average 42% reduction in PSA levels. Two of these four patients have PSA declines of 70-85% continuing beyond 3 months, as shown in Figures 13A and B. Mean time to PSA nadir was 7 weeks post-treatment (range: 2-13 weeks). Measurable responses have also been seen in these patients. The patient in whom the PSA level declined by 85% had 90% shrinkage of multiple soft tissue metastases. The patient with the 70% decline in PSA had a measurable decrease in soft tissue disease of 40%.

Conclusions:

[00382] ^{90}Y-DOTA-deJ591 is non-immunogenic (which would allow for repeated treatments) and toxicity has been limited to dose-related, reversible myelosuppression. Importantly, ^{90}Y-DOTA-deJ591 has dose-related anti-tumor effects in patients with advanced prostate cancer. Phase I data indicates that a single administration of ^{90}Y-DOTA-deJ591 (less than or equal to 20 mCi/m^2) is safe with optimal dosimetry for the treatment of prostate cancer. In addition, the radiation dosimetry estimates indicate that multiple administrations are also safe.

Example 11 - Evidence of PSA Responses in Prostate Cancer Patients Receiving ^{90}Y-deJ591

[00383] *Two patients receiving* ^{90}Y-DOTA-*deJ591 had rising PSA levels prior to* treatment with radiolabeled J591 (see Figures 13A and 13B). The X-axis on the plots represents time (in days). Negative numbers on this axis indicate days prior to J591 treatment. At the "0" time point, the patients received ^{90}Y-J591 for therapy. The graphs demonstrate that the rapidly rising PSA prior to treatment takes a sharp turn within a few weeks of treatment and becomes stable for a long period of time thereafter (at least ten weeks). The stability of the PSA level indicates that the progressive disease has stopped progressing. Higher doses of radiolabeled J591 may lead to a decrease in the disease burden and/or a prolongation of the cessation of tumor growth rate. In addition, repeated doses may also lead to absolute declines in the tumor burden as well as substantial prolongation of cessation of tumor growth rate.

Example 12 - Human Trial with ^{177}Lu-DOTA-deJ591; Phase I Trial of De-immunized mAb deJ591-DOTA-^{177}Lutetium In Patients With Relapsed Prostate Cancer

[00384] This example describes a clinical study of subjects who have prostate cancer that has relapsed after definitive therapy (e.g., surgery and/or radiation) and/or who are hormone independent and for whom no standard therapy exists. There is currently no curative therapy for these patients. Furthermore, the example focuses on ^{177}Lu labeled deJ591. ^{177}Lu is a both a beta- and a gamma-emitter. As such, it can be used for both radiotherapy and imaging.

[00385] The objectives of this trial were to: (1) define the toxicity and maximum tolerated dose (MTD) of de-immunized monoclonal antibody (mAb) deJ591-DOTA-^{177}Lutetium (^{177}Lu) in patients with prostate cancer who have recurrent and/or metastatic prostate cancer (Pca); (2) define the pharmacokinetics of deJ591-DOTA-^{177}Lu; (3) define the biodistribution and dosimetry of deJ591-DOTA-^{177}Lu; (4) define the human anti-de-immunized antibody (immune) response to deJ591-DOTA-^{177}Lu; and (5) define the preliminary efficacy (response rate) of deJ591-DOTA-^{177}Lu.

Treatment Protocol:

[00386] Patients received a dose of deJ591-DOTA-^{177}Lu administered at an infusion rate of $\leq$ 5mg/min. The total dose of deJ591 remained fixed at 10 mg/m^2. The ^{177}Lu dose (in mCi/m^2) was escalated in cohorts of three to six patients for each dose level (see Table 15

below). DeJ591-DOTA-^{177}Lu was labeled at a specific activity between 3-10 mCi/mg antibody to reach the defined dose of ^{177}Lu, with the balance brought up to 10/m^2 mg total deJ591 with "cold" deJ591. Dose escalation was withheld until at least three patients at the ongoing dose level had been followed for ≥ 6 weeks without serious hematologic toxicity. If any of the initial three patients at a dose level experience grade 1 or 2 hematologic toxicity by 6 weeks, escalation was withheld until recovery began or until 8 weeks of further monitoring and evaluation of toxicity had occurred. If any patient experienced grade 3 or 4 hematologic toxicity, at least six patients needed to be entered at that dose level and followed for a minimum of 8 weeks or until recovery begins prior to escalation. If, at any time, two instances of dose-limiting toxicity were observed at a given dose level, further entry at that dose level will be halted. In such a case, at least 6 patients should be entered at the prior dose level to aid in defining MTD (see "Toxicity" section below).

[00387] Patients who develop ≥ grade 2 allergic reaction while receiving ^{177}Lu-DOTA-deJ591 did not receive further deJ591 and were followed for toxicity.

Table 18

Dose Level	Total deJ591*	^{177}Lu Dose
1	10 mg/m^2	10 mCi/m^2
2	10 mg/m^2	15 mCi/m^2
3	10 mg/m^2	30 mCi/m^2
4	10 mg/m^2	45 mCi/m^2
5	10 mg/m^2	60 mCi/m^2
6	10 mg/m^2	75 mCi/m^2
7	10 mg/m^2	90 mCi/m^2
8	10 mg/m^2	105 mCi/m^2

*consisting of deJ591-DOTA-^{177}Lu at specific activity between 3-10 mCi/mg with the balance to 10 mg/m^2 total with "cold" deJ591.

[00388] Patients were followed for a minimum of 12 weeks after the deJ591-DOTA-^{177}Lu administration. If the patient's disease was stable or responding at 12 weeks after his treatment, he was followed until progression.

[00389] Except as noted in Table 16, follow-up was as described above in Table 14.

Table 16

Human anti-deimmunized Ab	Day of deJ591-DOTA-^{177}Lu Rx, post-Rx week 1, 2, 4, 8, and 12, then every 12 weeks until progression
Chem-7 (including Electrolytes, BUN, creatinine, glucose)	Day of deJ591-DOTA-^{177}Lu Rx, post-Rx week 1, 2, 4, and 8, then every 4 weeks until stable, and then every 12 weeks until progression
Liver panel (including: albumin, bilirubin (tot & dir), AST, ALT, (alkaline phosphatase)	Day of deJ591-DOTA-^{177}Lu Rx, post-Rx week 1, 2, 4, and 8, then every 4 weeks until stable, and then every 12 weeks until progression
LDH	Day of deJ591-DOTA-^{177}Lu Rx, post-Rx week 1, 2, 3, and 8, then every 4 weeks until stable, and then every 12 weeks until progression

^{177}Lu-deJ591 Imaging:

[00390] Total body images were obtained within 1 hour post-infusion (day 0) and at least 5 additional time points in the subsequent 2 weeks (e.g., days 1, 3, and 5, 10, 15). The gamma camera images were obtained using a dual head ADAC gamma camera fitted with an appropriate collimator. The percent injected dose (% I.D.) in major organs (heart, liver, spleen, kidneys, bone marrow, GI tract and bladder) was estimated by drawing regions of interest (ROI) and determining the relative counts in each organ and kinetics of wash out from each organ. SPECT studies were sometimes performed on the abdomen, pelvis and/or areas of suspected metastatic lesions. Where possible, using known standards of ^{177}Lu, percent injected dose in tumor was estimated per gram of tumor mass.

Toxicity:

[00391] NCI CTEP Common Toxicity Criteria (CTC), version 2 (April, 1999) was utilized. Since CTEP has standardized the CTC, the NCI does not require inclusion of the CTC within this document. All treatment areas have access to a copy of the CTC version 2.0. A copy may also be downloaded from the CTEP web site.

[00392] Three to six patients were entered (or will be entered) at each dose level. Dose escalation was withheld until at least three patients at the ongoing dose level had been followed for 6 weeks without hematologic toxicity. If any of the initial three patients at a dose level experience grade 1 or 2 hematologic toxicity by 6 weeks, escalation will be withheld until 8 weeks to further evaluate toxicity. If any patient experiences grade 3 or 4 hematologic toxicity,

at least six patients had to be entered at that dose level and followed for a minimum of 8 weeks prior to escalation. If, at any time, two instances of grade 3 or grade 4 toxicity were observed at a given dose level, further entry at that dose level would be terminated.

Definition of Dose Limiting Toxicity (DLT):

[00393] Hematologic toxicity: Grade 4 granulocytopenia (ANC < 500/ ul) for > 7 days or grade 4 thrombocytopenia (platelets < 10,000). Other toxicity: grade $\geq$ 3 non-hematologic toxicity attributable to ^{177}Lu-DOTA-deJ591.

Adverse Event Definition:

[00394] An adverse event is defined as any untoward medical occurrence in a research patient during a clinical trial or 4 weeks-posttreatment, regardless of causality. This includes clinical or laboratory findings, inter-current illness or an exacerbation or progression of a disease or a condition present at the time of entry (baseline). An adverse event is non-serious if it does not meet any of the serious criteria (see below).

Causality/Attribution:

[00395] All clinical adverse events and abnormal laboratory values were evaluated by for potential relationship to the experimental agent. The following categorizes of causality/attribution will be utilized: definite, probable, possible, unlikely, and unrelated.

[00396] Abnormal clinical laboratory values of clinical significance which were present at baseline and did not change in severity or frequency during the experimental therapy and/or which can reasonably be attributed to the underlying disease were evaluated by the investigator and recorded in the "unrelated" category. Such events, therefore, were not be considered in the evaluation of the safety of this agent.

Preexisting Conditions:

[00397] In this trial, a preexisting condition (that is, a disorder present before the adverse event reporting period started) is not reported as an adverse event unless the condition worsens or episodes increase in frequency during the adverse event reporting period.

Adverse Event Definitions:

[00398] Each adverse event was classified as serious or non-serious and/ expected or unexpected. An adverse event is classified as serious if it: it resulted in death; it was life-threatening (i.e., the encountered adverse event placed the subject at immediate risk of death; it does not apply to an adverse event which hypothetically might have caused death if it had been more severe); it required or prolonged in-patient hospitalization; it resulted in persistent or significant disability or incapacity; and it resulted in a congenital anomaly/birth defect.

Grading:

[00399] Toxicity was graded on a scale of 0-4 using either the Common Toxicity Criteria scales or the following:

(1) 0 = no toxicity.

(2) 1 = mild toxicity, usually transient, requiring no special treatment and generally not interfering with patient activity.

(3) 2 = moderate toxicity which may be ameliorated by simple therapeutic measures; impairs usual activities.

(4) 3 = severe toxicity requiring therapeutic intervention and interrupting usual activities. Hospitalization may or may not be required.

(5) 4 = life threatening toxicity which requires hospitalization.

(6) 5 = a fatal toxicity.

Criteria for therapeutic response:

[00400] Prostate cancer is manifest by rising PSA levels, new lesions on bone scan, new disease-related symptoms and, less commonly, increasing size of a measurable soft tissue mass. Response is commonly assessed either biochemically (PSA change) or by change in size of a measurable lesion/s.

[00401] Biochemical (PSA) response was determined as described above. In patients with measurable disease: Complete response is defined as complete disappearance of all measurable lesions by physical examination or imaging studies with no appearance of new lesions for > 2 months. Partial response: is defined as a 50% or greater reduction in the sum of the products of

the longest perpendicular diameters of all measurable lesions. There may be no new lesions. Stable disease: patients who do not meet the criteria of partial response and who are without signs of progressive disease for > 2 months. Progressive disease is defined as a greater than 25% increase in the sum of the products of the longest perpendicular diameters of the immeasurable lesions, the appearance of new lesions or a rise in prostate specific antigen above pre-treatment baseline.

[00402] Duration of response: Typically, the first sign of progression will be a rise in serum PSA. In this trial the duration of response will be the time interval from treatment initiation until progression is documented by either a rise in PSA, enlargement of the measurable lesion/s, or new lesion/s on bone scan. The rising PSA must be confirmed by a second, serially rising PSA and the duration will be defined as the time from initiation of treatment to the time of the first rising PSA.

Results:

[00403] Hormone refractory patients with CT/Bone scan documented prostate cancer lesions and increasing PSA levels were enrolled in a Phase I dose-escalation (10-75 mCi/m^2) study. All of the patients had failed one or more forms of hormone therapy.

[00404] To date, nineteen patients (three groups of patients, 3-6/group) have received ^{177}Lu-DOTA-deJ591 (10 mg/m^2). Each group received a different dose of ^{177}Lu-DOTA-deJ591: 10, 15, 30, 45, or 60 mCi/m^2 (0.37, 0.74, 1.11, 1.48, or 1.85 GBq, respectively). Blood samples were obtained for two weeks, and imaging studies were performed five times during the same two weeks. Blood chemistry, hematology, and PSA levels were monitored for three months or longer.

[00405] Imaging studies showed specific tumor localization of ^{177}Lu-DOTA-deJ591. Four patients had previously unrecognized metastatic foci demonstrated upon 177Lu-DOTA-deJ591 imaging, which was subsequently confirmed by conventional imaging. The radiation dosimetry estimates show that the liver is the critical organ receiving 7.6+/-3.3 rads/mCi. Dose to bone marrow based on blood activity is 0.7 rads/mCi. Plasma T1/2 of ^{177}Lu-DOTA-deJ591 was 43+/-11 hours. No significant changes were observed in blood chemistry, or liver or kidney function. Hematological changes were observed at different dose levels, but even at the 60 mCi/m^2 dose level, no serious toxicity was observed.

[00406] The first nine patients that entered the study received a dose of ^{177}Lu-DOTA-deJ591 at levels of 10 mCi/m^2 (three patients), 15 mCi/m^2 (three patients), and 30 mCi/m^2 (three patients). All patients had failed one or more forms of hormone therapy, and one (17%) patient had failed at least one chemotherapy regimen. Toxicity was minimal, with no patients having grade 2 or 3 adverse events, and limited to reversible myelosuppression, primarily thrombocytopenia. No patients developed an immune response to ^{177}Lu-DOTA-J591.

[00407] Among these first nine patients, dose-related anti-tumor effects were noted. Following treatment at the 10 mCi/m^2 dose level, two patients had PSA levels that continued to rise despite treatment, while the remaining patient showed stabilization of PSA levels. At 15 mCi/m^2, two patients had a mean decrease of 35% in PSA levels, while one patient's PSA levels progressed despite therapy. Mean time to PSA nadir was 4 weeks post treatment (range: 2-6 weeks). One of the three patients that exhibited a decrease in PSA levels, who did not have measurable disease, continued to have 50% reduced PSA levels even at 18 weeks post-treatment (see Figure 14). Some of these patients are currently being retreated and have received at least three doses of ^{177}Lu-DOTA-deJ591.

Conclusions:

[00408] ^{177}Lu-J591 is non-immunogenic (which would allow for repeated treatments) and has low toxicity at doses used thus far. It has dose-related anti-tumor effects in patients with advanced prostate cancer. In contrast to ^{90}Y-DOTA-deJ591, which has a maximum tolerated dose estimated to be about 20 mCi/m^2, ^{177}Lu-DOTA-deJ591is safe even at a 30 mCi/m^2 dose level. ^{177}Lu-DOTA-deJ591 appears to eliminate disadvantages associated with both ^{131}I-DOTA-deJ591 (which is dehalogenated in vivo and is not ideal for mAbs that are internalized) and ^{90}Y-DOTA-deJ591. The longer residence time of ^{177}Lu-DOTA-deJ591 in tumor tissue may also augment the anti-tumor response.

Example 13 - Imaging Non-Prostate Cancers

[00409] In addition to prostate epithelial cells, immunohistochemical studies show that PSMA is also expressed by vascular endothelial cells of numerous solid tumors, but not by normal vascular endothelium in benign tissues. As discussed above, this expression pattern of PSMA occurs in virtually all solid tumors.

[00410] An IRB approved Phase I dose escalation trial of ^{111}In-DOTA-deJ591 was initiated to assess its value as an therapeutic agent for vasculotoxic therapy, to define its toxicity and maximum tolerated dose, to determine its pharmacokinetics and biodistribution, and to assess for immunogenicity. Eligible patients were those with refractory solid tumor malignancies whose tumor types are known to express PSMA on the neovasculature.

[00411] Fifteen patients received 5mg (three patients), 10mg (six patients), or 20mg (six patients) of ^{111}In-DOTA-deJ591, followed by a second dose 14 days later.

[00412] Patients the participated in the study included eight renal cell cancer patients, four bladder cancer patients, two colon cancer patients, and one pancreas cancer patient. All patients underwent whole body and SPECT imaging on days 0 (the day of injection), 2, 5, and 7 of ^{111}In-DOTA-deJ591 injection. The ^{111}In-DOTA-deJ591 imaging results were compared with CT and bone scans.

[00413] Imaging data revealed ^{111}In-DOTA-deJ591 tumor targeting in ten of the fifteen patients (7 renal cell cancer, 2 bladder cancer, and 1 colon cancer). Targeting was best observed on days 2 and 5, and was independent of antibody mass delivered. No additional sites were detected on SPECT. Targeted metastatic sites included: lungs, femur, retroperitoneal and cervical lymph nodes. Brain metastasis in one patient (with renal cell cancer) was first detected by ^{111}In-DOTA-deJ591 imaging and later confirmed by MRI. ^{111}In-DOTA-deJ591 imaging was false negative in five of the fifteen patients (2 bladder cancer, 1 colon cancer, 1 pancreas cancer, and 1 renal cell cancer). Non-targeted metastatic sites included: liver, renal bed, pancreas, lungs, and celiac lymph nodes. Undetected lung lesions measured less than 1cm in size.

[00414] No objective responses occurred, although a colon cancer patient had a 50% decline in CEA and two patients had improvement in cancer pain and performance status.

[00415] Two different patients with metastatic kidney cancer, having disease that had spread to the lungs, lymph nodes, and/or bones, were injected with deJ591 labeled with ^{111}Indium. Images of the patients, taken within 2 days of injection, demonstrated significant uptake of the antibody in the known tumor sites in these varying tissues and organs. The extent of the uptake of antibody is both substantial and rapid.

[00416] Among physiological sites, highest uptake was observed in the liver. Plasma clearance and liver uptake were dependent upon antibody mass; lower mass resulted in faster plasma clearance and higher liver uptake (in terms of percentage uptake). Plasma clearance

(T1/2) for 5mg, 10mg, and 20mg of deJ591 were 21+/-11 hours, 24+/-6 hours, and 37+/-8 hours, respectively. Liver uptake for the same dose levels was 28%+/-14%, 17%+/-7%, and 13%+/-5%, respectively.

[00417] Based on these data, the protocol was revised to provide dosing for 6 consecutive weeks (10, 20, 40 and 80 mg/week dose levels) with the option for re-treatment on 8 week cycles if patients have stable or responding disease. To date, nine patients are currently receiving treatment on this schedule.

[00418] ^{111}In-DOTA-deJ591 specifically targets vascular endothelium of solid tumors. These trials demonstrate that deJ591 is an effective approach to targeting solid tumor vascular endothelium with radioactivity or cytotoxins.

Example 14 - Phase II Trial of mAb deJ591 in combination with Low-dose Subcutaneous Interleukin-2 in Patients with Recurrent Prostate Cancer

[00419] The subjects for this trial have prostate cancer which has relapsed after definitive therapy (e.g. surgery and/or radiation) and/or who are hormone independent and for whom no standard therapy exists. There is currently no curative therapy for these patients.

[00420] The objectives of the trial are to: (1) To define the preliminary efficacy (response rate) of mAb deJ591 in combination with daily low-dose subcutaneous IL-2 in patients who have recurrent and/or metastatic prostate cancer; (2) to study the toxicity of mAb deJ591 in combination with daily low-dose subcutaneous IL-2; and (3) to measure in vitro the effect of IL-2 on the immune response.

Low-Dose IL-2 Therapy:

[00421] IL-2 promotes the proliferation and enhances the secretory capacity of all major types of lymphocytes, including T cells, B cells, and natural killer (NK) cells (Smith K.A. (1988) *Science* 240:169). The IL-2 stimulated expansion of antigen-selected T-cell and B-cell clones determines the magnitude of antigen-specific immune responses, while the quality of the response is determined by IL-2 promoted secretion of additional cytokines, cytolytic molecules and antibodies (Smith K.A (1993) *Blood* 81:1414-1423). In addition, through its effects on NK cells, IL-2 stimulates antigen-nonspecific host reactions that involve an interplay between NK cells and monocytes. As a result of these functions, it follows that IL-2 should be useful as an

immune stimulant, particularly in cancer immunotherapy. The therapeutic use of IL-2, however, is made difficult because one of its major effects consists of the stimulation of secondary cytokine secretion by IL-2-responsive cells. Many of the potential beneficial effects of IL-2 can be attributed to these secondary cytokines that recruit and activate additional cell types, especially monocytes, that contribute to the total immune/inflammatory reaction. However, these same secondary cytokines, when produced in too large amounts, can also lead to severe toxicity. IL-2 was first used in very high doses for the treatment of cancer, equivalent to 150 million units (MU) of Chiron Corporation IL-2 (Rosenberg S.A (1990) *Sci. Am* 262:62-69). This high dose was determined using the dose-escalation and dose-intensification principles of chemotherapy, and was associated with significant grade 3 and 4 toxicity. IL-2 in high doses is known to cause serious side effects including fever, rigors, malaise, myalgia, nausea/vomiting, hypotension and possibly death.

[00422] In the 1990s, researchers began to examine the immunomodulatory effects and toxicities of continuous low dose IL-2 (Smith K.A (1993) *Blood* 81:1414-1423; Caligiuri et al. (1991) *J Clin Oncol* 9:2110-2119; Caligiuri *et al.* (1993) J Clin Invest 91:123-132; Bernstein et al. (1995) *Blood* 86:3287-3294). These studies demonstrated that doses of IL-2 as low as 1.2 MU daily resulted in the specific expansion of NK cells with minimal toxicity. Bernstein et al. (1995) *Blood* 86:3287-3294; Lalezari et al. (2000) HIV Clin Trials). Potential side effects include injection site reactions (usually redness at the injection site), asthenia, flu-like symptoms, nausea, diarrhea and eosinophilia. The selective expansion of human $CD3^-$, $CD56^+$ NK cells during low-dose IL-2 begins within 2 weeks of therapy and plateaus after 4-6 weeks of treatment (Smith K.A (1993) *Blood* 81:1414-1423; Fehniger et al. (2000) *J Clin Invest* 106:117-124). NK cells can account for as many as 60%-80% of PBMCs after one month of therapy (Smith K.A (1993) *supra*). Recent studies suggest that increased NK cell number results from enhanced NK-cell differentiation from bone marrow progenitors, combined with a delay in IL-2 dependent NK-cell death (Fehniger et al. (2000) *supra*). The low-dose IL-2 regimens have been specifically designed to completely avoid toxicity.

Combination Monoclonal Antibody and IL-2 Therapy:

[00423] The combination of monoclonal antibodies and IL-2 potentially should enhance monoclonal antibody efficacy. IL-2 will function to augment the reticuloendothelial system to

recognize antigen-antibody complexes by its effects on NK cells and macrophages. Thus, by stimulating NK cells to release IFN, GM-CSF, and TNF, these cytokines will increase the cell surface density of Fc receptors, as well as the phagocytic capacities of these cells. Therefore, the effector arm of both the humoral and cellular arms will be artificially enhanced. The net effect will be to improve the efficiency of monoclonal antibody therapy, so that a maximal response may be obtained. A small number of clinical trials have combined IL-2 with a monoclonal antibody (Albertini et al. (1997) *Clin Cancer Res* 3:1277-1288; Frost et al. (1997) *Cancer* 80:317-333; Kossman et al. (1999) *Clin Cancer Res* 5:2748-2755). In such studies, IL-2 was administered intravenously by either bolus or continuous infusion. Toxicity was associated with higher doses of IL-2.

IL-2 Therapy in Prostate Cancer:

[00424] A variety of studies have examined the effects of IL-2 on prostate cancer cells *in vitro* and in prostate cancer animal models, Moody et al. Interleukin-2 transfected prostate cancer cells generate a local antitumor effect *in vivo* (*Prostate*, 24: 244-251, 1994; Sokoloff et al. (1996) *Cancer*, 77: 1862-1872; Triest et al. (1998) *Clin Cancer Res*, 4: 2009-2014; Hautmann et al. (1999) *Anticancer Res*, 19: 2661-2663; Hillman et al. (1999) *Cancer Detect.Prev.* 23: 333-342), although there have been few clinical trials of IL-2 in patients with advanced prostate cancer. Hillman et al. (1999) *supra*; Maffezzini et al. (1996) *Prostate*, 28: 282-286; Morris et al. (2000) *Cancer*, 89: 1329-1348). A Phase II trials of I.V. intermediate dose IL-2 (dose ranging from 10-15 MU daily x 4) in patients with hormone refractory prostate cancer was conducted. In six out of ten patients, a transient decline in PSA levels was observed. It is unclear if this was from anti-tumor activity, or if IL-2 affected PSA expression, as had been reported *in vitro* studies using LNCaP cells (Sokoloff et al. (1996) *supra*). No regression of measurable disease was observed in any patient. The effect of daily sub-cutaneous low-dose IL-2 in patients with progressive prostate cancer has not been examined.

Specific Aims:

(1) To define the preliminary efficacy (response rate) of mAb deJ591 in combination with daily low-dose subcutaneous IL-2 in patients who have recurrent and/or metastatic prostate cancer.

(2) To study the toxicity of mAb deJ591 in combination with daily low-dose subcutaneous IL-2.

(3) To measure the effect of IL-2 on the peripheral blood mononuclear cell (PBMC) population.

(4) To measure in vitro the effect of IL-2 activated NK cells from patients on this protocol to induce ADCC with mAb deJ591.

Treatment Protocol:

[00425] Patients receive daily low-dose subcutaneous rIL-2 (1.2×10^6 IU/m^2/day) on day 1 through day 56. After three weeks of IL-2 administration, patients receive deJ591 by I.V. (25 mg/m^2) once a week for three consecutive weeks (on days 22, 29, and 36). IL-2 administration is continued during this period another two weeks afterwards. This 8-week regimen constitutes one cycle of therapy. Patients are evaluated for response at the end of one cycle. Patients who have responded to therapy or have stable disease are eligible for additional cycles of therapy. Additional cycles will be initiated when there is at least two consecutive rises in PSA at least 2 weeks apart. A three-week lead in with IL-2 should be sufficient to significantly increase the NK cell population. The dose of deJ591 is based on preliminary pharmacokinetic data from the Phase I ^{111}In-labeled deJ591 trial. The dose of antibody will be adjusted based on additional data analysis of patients treated in this manner.

[00426] A single cycle of treatment has been initiated. Patients who progress by radiographic documentation after one cycle will be removed from the study. Patients who responded by radiographic documentation, or who had stable disease by imaging with >50% decline in PSA value, or who had stable disease by imaging with stable PSA levels, will be examined every 3-4 weeks. Responding or stable patients will be eligible for re-treatment (a second 8-week cycle) following 2 consecutive PSA rises at least 2 weeks apart, at the discretion of the Principal Investigator and the option of the patient. Patients who have stable disease by imaging with a rising PSA (>25% of pre-treatment value) will be examined every 3-4 weeks. Re-treatment will be at the discretion of the Principal Investigator. In order to be retreated, the patient must have a immune reaction titer <1/100 and satisfy all initial Eligibility and Exclusion criteria. Re-treatment will be at the identical dose formulation as the initial dose given to the patient.

Patient Selection:

[00427] The trial is a pilot study to ascertain if low-dose IL-2 in combination with deJ591 has activity in prostate cancer. It is possible that low-dose IL-2 may have activity in prostate cancer patients. However, it has been decided not to treat patients with IL-2 alone, or delay antibody therapy, if the PSA declines after 3 weeks of therapy on low-does IL-2, as it will be interesting to study the combined effect of deJ591 with IL-2. If significant activity is observed, a subsequent randomized trial of IL-2 alone vs. IL-2 with deJ591 will be conducted. It is unclear which stage of patient with prostate cancer might benefit from this approach. Therefore, at least ten patients will be treated, each in the following subgroups: 1) Biochemical relapse, hormone naive: rising PSA following radical prostatectomy or radiation therapy, without evidence of metastatic disease; 2) Biochemical relapse, hormone refractory: rising PSA following hormonal therapy without prior chemotherapy (these patients may or may not have radiographic documented metastatic disease); and 3) Hormone refractory, having received prior chemotherapy. About 30-40 patients will be enrolled on this trial.

Toxicity:

[00428] Toxicity will be scored using the Cancer Therapy Evaluation Program Common Toxicity Criteria, Version 2.0 (April 1999).

IL2 Dosage modifications:

[00429] IL-2 will be permanently discontinued for any study drug-related Grade 4 toxicity except hematologic (which can be corrected with EPO or G-CSF or blood transfusions). If at any time during the study, ALT is $\geq$ 20 times the upper limit of normal, both IL-2 and mAb therapy will be permanently discontinued in that subject. Electrolyte abnormalities that can be readily corrected will not require permanent drug discontinuation.

[00430] This protocol allows for one dose level reduction of IL-2 of 25% to 0.9 mU/M^2. Subjects receiving IL-2 may have their IL-2 interrupted for $\geq$ Grade 1 toxicity for 24-48 hours. Toxicities that may cause IL-2 to be temporarily interrupted are:

Electrolyte abnormalities Grade 1 or higher that cannot be rapidly corrected;

Grade 1 or higher respiratory toxicity;

Grade 3 local reaction or any local reaction involving ulceration;
Fever > 38 oC, or intolerable flu-like symptoms or rigors;
Other Grade 3 or greater toxicity, either related or unrelated to IL-2;
Fever suspected to be related to an opportunistic infection;
Grade 1 fatigue; and
Grade 2 hematologic toxicity that can be corrected with EPO, G-CSF, or blood transfusions.

[00431] Subjects who have their IL-2 interrupted can have it resumed at the same dose or at one dose level reduction within 24-48 hours of stopping drug. The dose of IL-2 for that subject may later be increased to the initial dose at the discretion of the subject and the local investigator.

MAb deJ591:

[00432] Allergic events will be managed as follows: rash, pruritis, urticaria and wheezing will be treated with benadryl and/or steroids as clinically appropriate. Anaphylaxis or anaphylactoid signs or symptoms will be treated with steroids and/or epinephrine as clinically indicated. Patients will be treated in a general clinical research center equipped for cardiopulmonary resuscitation.

[00433] Both drugs will be discontinued in patients who experience any grade 3 or 4 toxicity during the three weeks when mAb is administered. Treatment with mAb will resume at a 25% reduction in dose of mAb deJ591 after the toxicity has returned to grade I or less. If grade 3 or 4 toxicity recurs on attenuated doses, mAb treatment will be discontinued. IL-2 treatment will continue for completion of the 8-week cycle.

Criteria for therapeutic response:

[00434] Response will be assessed either biochemically (PSA change) and/or by change in size of a measurable lesion/s using standard response criteria for prostate cancer (Dawson N.A. (1999) *Semin Oncol* 26:174-184; Bubley, G.J., et al. (1999) *J Clin Oncol*, 17:3461-3467)

[00435] Biochemical (PSA) response will be determined as described above. Criteria for measuring the disease is as follows: Complete response is defined as complete disappearance of

Statistical Methods:

[00439] For all outcome variable with measurements taken at various time points, a Repeated Measures Analysis of Variance (RMANOVA) will be carried out to determine any patterns of change over time. Upon finding a significant time effect, Bonferroni- adjusted painwise contrasts will be calculated to determine which time points differ from one another. To simplify the analysis for data obtained at multiple time points, one may choose to " aggregate" the measurement values taken over time. An area under the curve (AUC) analysis for the PSA levels can be carried out. Alternatively, a "slope analysis" may be carried out.

Current Status of Results:

[00440] Six patients have been entered and three have completed 8 weeks of therapy; two patients are being retreated based on PSA stabilization. A third patient had objective disease progression. Toxicities have been expected and minor including fatigue, injection site reactions and asymptomatic thyroid function abnormalities. IL-2 mediated immune modulation is being evaluated by flow cytometry on peripheral blood to quantify expression of lymphocyte subsets pre- and post-mAb treatment.

Example 15: Conjugation of deJ591 to the maytansinoid cytotoxin DM1

[00441] This example describes a process for the production of the deJ591-DM1 immunoconjugate. The process is based on standard methods known in the art and can therefore be generalized to other antibodies, includeing other antibodies of the invention such as deJ415.

[00442] The methods of conjugation are based on several small scale experiments, including one experiment performed using 5g of deJ591 starting material (Lot 1552-60S) and three experiments performed using between 6.7g and 7.3g of deJ591 starting material (Lots 1552-168, 1552-104, and 1610-036).

[00443] The steps involved in the methods of conjugation are as follows:

1) 5g to 7.5g of deJ591 antibody is concentrated by tangential flow filtration (10kD NMWCO membranes) to 25-30 mg/ml and diafiltered against 5 volumes of 50mM potassium phosphate, 2mM EDTA, pH 6.0. The yeild is typically between 98% and 100%.

2) The concentrated antibody is filtered through a 0.2 μ filter, if opalescent, and then modified with N-succinimidyl 4-(2-pyridyldithio)propionate (SPP) at a concentration of 20-22 mg/ml antibody and 7 molecules of SPP per molecule of antibody. The modification is

done in 50mM potassium phosphate, 2mM EDTA, 5% ethanol, pH 6.0, for 2.5 +/- 0.5 hours. The modification vessel is a 500ml round bottom flask.

3) The modified antibody is separated from the reaction mixture of step 2) using gel filtration chromatography and a Sephadex G-25™ column. The column load represents about 25% of the column volume and the chromatography is done in 50mM potassium phosphate, 2mM EDTA, pH 6.0, at a flow rate of 50 cm/hr. The modified antibody elutes between 38-75% column volume. Typically the yeild of this step between 95% and 100% and the SPP to antibody ration is about 5.4 to 5.9 SPP molecule/antibody.

4) At a concentration of about 10mg/ml, the modified antibody is conjugated with DM1 (using 1.7 molecules of DM1/molecule of SPP conjugated to the antibodies) for 20 +/- 4 hours. Typically, the reaction time is between 16.25 and 17.7 hours and is carried out in a 1L round bottom glass flask equipped with a magnetic stirring bar. The conjugation reaction is done in 3% DMA, 10% sucrose (100mg sucrose/ml of reaction). At the end of the reaction the conjugated antibody is filtered through a 2.0 μ filter and a spectrophotometric reading is taken.

5) The conjugated antibody is seperated from unreacted DM1 by gel filtration chromatography using a Sephadex G-25™ column. The column load represents 22-23% of the column volume and the flow rate is about 50 cm/hr. The column is equilibrated and run in 20mM succinate, 5% sucrose (50mg/ml), pH 5.5. The antibody conjugate elutes between about 31% and 65% of column volume, and is collected from the start of the peak elution to the start of the peak trailing edge as a single fraction, followed by fractionation of the remaining peak material in 15x2% column volume fractions. All fractions are adjusted to 100mg/ml of sucrose (10% sucrose) through the addition of appropriate amounts of 50% sucrose. The 2% column volume fractions are assayed by analytical sizing (TSK 3000SWL) and selected fractions (fractions 1 and 2) are pooled together with the main peak. The fractions are assayed using analytical sizing with the pooling criterion being the 24 minute peak representing <20% or the total peak area. Typically the yield of this step is between 60% and 65% with the exception of run 1552-104 where there was no sucrose present in the reaction and/or purification mixture. The eluted antibody concentration ranges from 3.8 to 4.2 mg/ml and the ration of DM1/antibody ranges from 3.6 to 3.9.

6) The antibody conjugate is then concentrated to 7-10 mg/ml using a 10kD NMWCO tangential flow filtration membrane and diafiltered against 5 volumes of 50mM

succinate, 10% sucrose, pH 5.5 (Inlet Pressure < 10 psi). Following diafiltration the antibody conjugate is adjusted to 5 mg/ml. Typical yield for this step is between 92% and 100%, with the final protein concentration being between 4.85 and 5.1 mg/ml.

7) Finally, the antibody conjugate is filtered through a 0.2 m filter and aliquoted to the specified volumes. Step yield is between 90% and 100% and the final DM1-antibody ratio is 3.5 to 3.8.

[00444] The resulting deJ591-DM1 conjugates were analyzed according to appearance, concentration, DM1/antibody ratio, endotoxin, non-specific cytotoxicity, acetone extractable DM1, analytical sizing, reduced and non-reduced SDS-PAGE, pH, bioburden, specific cytotoxicity, and IEF. Selected analytical results for lots 1552-168, 1552-104, and 1552-036 are shown in Table 19, below.

Table 19

Lot No.	Amount Recovered	Concentration (mg/ml)	DM1/antibody	Process Recovery (%)
1552-168	3.85	5.1	3.7	57.2
1552-104	2.75	4.85	3.5	37.8*
1610-036	3.41	5.05	3.8	47.2
Mean	3.34	5.00	3.67	47.4
Standard Dev.	0.55	0.13	0.15	9.7
% c.v.	16.6	2.6	4.2	20.5

* Lower recovery due partly to the lack of sucrose in the conjugation reaction and the second G-25 gel filtration run and partly to the fact that the front end of the product peak was not collected due to a malfunction in the chart recorder.

Example 16: Use of the mAbs for targeted delivery of cytotoxic drugs to prostate cancer cells

[00445] Anti-PSMA antibodies can be conjugated to substances with high cytotoxic potential, such as drugs of the maytansinoid class. Maytansinoids exert their cytotoxic effects by interfering with the formation and stabilization of microtubules. They have 100- to 1000-fold greater cytotoxic potential than conventional chemotherapeutic agents (such as doxorubicin, methotrexate, and Vinca alkaloids) (Chari, R.V.J. et al. (1992) *Cancer Res.* 52: 127).

[00446] Both murine and deimmunized J591 antibodies have been conjugated to the maytansinoid, DM1, via a hindered disulfide bond. This bond is cleaved intracellularly allowing release of the drug. One or more lysine residues in the constant regions of the antibodies were conjugated to a linker containing a pyridyldithio group, which was, in turn, coupled to a maytansinoid toxin. A ratio of 3 to 4 moles of maytansinoid per mole of IgG is preferred.

[00447] The process for the DM1-linked J591 antibodies starts by reacting J591 with a linker that contains both a pyridyldithio group and a N-hydroxysuccinimide leaving group. In this case, the linker was N-succinimidyl 4-(2-pyridyldithio)propionate (or SPP), although other linkers can be used. The products of the reaction include modified J591 antibodies that contain one or more linker groups (4-(2-pyridyldithio)propionone) attached to surface exposed lysine groups, with the linker groups retaining the pyridyldithio reactive groups, and N-hydroxysuccinimide leaving groups. The J591 antibodies are then separated from the reaction mixture and N-hydroxysuccinimide by gel filtration, e.g., using sephadex G25. The modified J591 antibodies are reacted with DM1, which contains a thiol group that reacts with the pyridyldithio groups now present on the surface of the modified antibody, thereby producing J591-DM1 immunoconjugates and thiopyridine. The J591-DM1 immunoconjugate is isolated from the reaction mixture and thiopyridine by size exclusion chromatography, e.g., using a sephacryl S300 column. Methods for preparing maytansinoid conjugates are described in US Patent Nos. 5,208,020; 5,475,092; 5,585,499; 5,846,545; and 6,333,410, the contents of which are incorporated by reference.

[00448] A study evaluating the efficacy, toxicity, and antigen selectivity of the murine J591-DM1 immunoconjugate in the treatment of prostate cancer cells *in vivo* is described below.

Experiment 1: Establish Efficacy, Toxicity, and Dose-Response Curves In-Vivo

[00449] Tumor xenografts of LNCaP cells were established in the right flank of BALB/c mice (5×10^6 cells injected subcutaneously). Animals were observed until tumors were visible (7 - 10 mm). Animals were then treated with PBS, unconjugated mAb J591, unconjugated DM1, both mAb J591 and DM1 but unconjugated, and J591-DM1 immunoconjugate (100, 200, 300, and 400 mcg/day intraperitoneal qday x 5 days). The mAb J591 was modified to introduce dithiopyridyl groups and then conjugated to DM1 via a hindered disulfide bond, as described above. Unconjugated mAb J591 and DM1 were given in equimolar concentrations.

Experiment 2: Selectivity for PSMA-positive tumors

[00450] Tumor xenografts of both LNCaP cells (5 x 10^6 cells injected subcutaneously in the right flank) and PC3 cells (3 x 10^6 cells injected subcutaneously in the left flank) were established in BALB/c mice. Animals were observed until LNCaP tumors were visible (PC3 tumors were present but much smaller). Animals treated with J591-DM1 immunoconjugate: 300 mcg/day intraperitoneal qday x 5 days

Experiment 3: Determine optimal dosing schedule for the immunoconjugate

[00451] Tumor xenografts of LNCaP cells were established subcutaneously in the right flank of BALB/c mice. When tumors were visible, animals were treated with PBS, unconjugated mAb J591, J591-DM1 immunoconjugate (300 mcg/day intravenously qday x 5 days; one course given), J591-DM1 immunoconjugate (400 mcg/day intravenously every other day for 5 doses in 10 days; one course given), and J591-DM1 immunoconjugate (300 mcg/day intravenously qday x 5 days; two courses given for a total of 10 doses). For animals receiving 2 courses of J591-DM1 immunoconjugate, the second 5-day course was given at tumor volume nadir (typically seen on days 21 - 24 after completion of the first course).

[00452] All animals were photographed pre- and post-treatment and tagged for identification. Tumor volume was determined (length x width x depth) and closely followed. Animal weight was followed as a measure of toxicity.

Results: Experiment 1:

[00453] All mice treated with unconjugated DM1 (either alone or with unconjugated mAb J591) died within 48 hours of treatment completion. Immunoconjugate at doses of 400 mcg/day was lethal to 50% of the mice. With doses of 300 mcg/day, significant reductions in tumor volume were noted with several complete responses and minimal toxicity. These complete responses were not durable, however, with subsequent increases in tumor size noted 2 - 20 days after tumor volume nadir.

Results: Experiment 2:

[00454] In mice with both LNCaP and PC3 tumors, LNCaP tumor volume decreased substantially after treatment with the J591-DM1 immunoconjugate. Although complete responses were not achieved in these animals, tumor volume nadirs averaged 0.08 cm^3. PC3 tumor growth was not affected by the J591-DM1 immunoconjugate as noted by a steady increase in PC3 tumor volume after treatment.

Experiment 3:

[00455] Although route of administration was different (intravenous vs. intraperitoneal), treatment with one course of J591-DM1 immunoconjugate paralleled the results obtained from Experiment 1. Complete responses were achieved (at days 21 - 24) but these results were not durable. In animals treated with every other day dosing of 400 mcg, toxicity was demonstrated (weight loss > 10 % of total body weight) but all animals eventually survived. Tumor volume decreased in all animals but complete responses were not achieved. All animals treated with 2 courses of J591-DM1 immunoconjugate have achieved complete responses lasting at least through day 66.

Conclusions:

[00456] Murine J591 anti-PSMA antibody can be conjugated effectively to drugs of the maytansinoid class (such as DM1). These J591-DM1 immunoconjugates provide highly selective, antigen-specific targeted delivery of this cytotoxic drug to PSMA-positive prostate cancer cells in-vivo. The greatest reduction in tumor volume with minimal toxicity was noted at a dose of 300 mcg/day. The optimal dosing schedule appears to be two 5-day courses of J591-DM1 immunoconjugate with the second course given at tumor volume nadir (day 21 - 24).

Example 17 – Pharmacodynamics and Efficacy of the deJ591-DM1 immuoconjugate

[00457] In parallel to the experiments described in Example 16, additional experiments were performed with the deJ591-DM1 immunoconjugate. The experiments and results obtained therefrom are described below.

In vitro pharmacodynamics of deJ591-DM1 and DM1:

[00458] Experiment: LNCaP cells were plated into 96 well plates at an initial density of 1000 cells per well for a proliferation assay. deJ591-DM1 or DM1 was added to the wells over a range of concentrations (0.01 nM to 100 nM) and left in contact with the cells for defined periods of time (0.5, 2, 8, 24, 72 and 144 hours). At the end of that period the drug containing media was removed and replaced with drug free media until a total of 144 hours elapsed from the start of the experiment. The resulting effect of drug exposure on proliferation was then determined.

[00459] Results: The pharmacodynamic analysis using the methodology developed by Kalns et al. (1995), Cancer Research 55:5315-5322, the contents of which are incorporated herein by reference, indicates that when considering the relative importance to observed effect from the variables (1) time of exposure and (2) concentration, that exposure time is more important for deJ591-DM1 compared to DM1, where concentration it the more important variable.

Serum levels of DEJ591-DM1 in mice after a single IV dose

[00460] Experiment: Mice were administered an intravenous 14.5 mg/kg dose of deJ591-DM1 and sera samples collected for analysis of the test article using an ELISA assay for human antibody. Samples were collected from groups of 3 mice at 6, 24, 48, 72 and 168 hours. Data were fit to a bi-exponential equation to determine pharmacokinetic parameters. These parameters were used to simulate serum levels after multiple dosing.

[00461] Results: Analysis of the phamacokinetic data indicated a serum half life of deJ591-DM1 in mice as measured in the ELISA assay, to be 130 hours.

Effect of dose interval on efficacy against CWR22 prostate xenografts

[00462] Experiment: Male SCID mice were implanted by serial passage of CWR22 prostate tumor xenograft. When these tumors reached 200 – 250 mm^3 size (estimated from external caliper measurement), mice were randomized into treatment groups of 8 to receive vehicle only (every 7 days) or deJ591-DM1 at a dose of 14.5 mg/kg antibody conjugate (equivalent to 240 ug/kg DM1) given on one of the following schedules (every 7, 14, 21, or 28 days). All treatments were given intravenously. Tumor growth and animal health were continually monitored throughout the study with tumor growth measured every 3 days.

[00463] Results: Differences in the schedule of administration have distinct effects on the observed tumor growth for the CWR22 xenograft in SCID mice. The tumor growth can be characterized as slowed growth for the schedule of 21 or 28 day dosing interval until they reached the maximum size permitted under our IACUC regulations. For the 7 and 14 day dosing interval schedule, there is an apparent block in tumor growth with a resumption of normal growth kinetics approximately 30 days after the last dose. The results when considered with the pharmacokinetic simulation suggest a relationship between duration of exposure and maintaining a minimum effective concentration.

Efficacy of deJ591-DM1 compared to the unconjugated antibody (deJ591) or the unconjugated tumor inhibitory agent (DM1)

[00464] Experiment: Male SCID mice were implanted by serial passage of CWR22 prostate tumor xenograft. When these tumors reached 200 – 250 mm^3 size (estimated from external caliper measurement), mice were randomized into treatment groups of 8 to receive vehicle only, deJ591-DM1 at a dose of 14.5 mg/kg antibody conjugate (equivalent to 240 ug/kg DM1), deJ591 at the same dose as deJ591-DM1, or DM1 given at a dose of 240 ug/kg. All treatments were given intravenously and on a schedule of every three days for 5 doses. Tumor growth and animal health were continually monitored throughout the study with tumor growth measured every 3 days.

[00465] Results: The unconjugated anti-PSMA antibody (deJ591) had no significant effect on reduction in the rate or extent of CWR22 xenograft tumor growth. DM1 administered as free drug produced some tumor growth delay, but was a minor response compared to the deJ591-DM1 administered on the same schedule with the same molar equivalent of the active DM1. The deJ591-DM1 produced a suppression of tumor growth for approximately 20 days following the last administered dose.

Efficacy of DEJ591-DM1 at different doses

[00466] Experiment: Male SCID mice were implanted by serial passage of CWR22 prostate tumor xenograft. When these tumors reached 200 – 250 mm^3 size (estimated from external caliper measurement), mice were randomized into treatment groups of 8 to receive vehicle only, deJ591-DM1 at a dose of 14.5 mg/kg antibody conjugate (equivalent to 240 ug/kg

DM1), or a lower dose of 7.25 mg/kg. All treatments were given intravenously and on a schedule of every seven days for 5 doses. Tumor growth and animal health were continually monitored throughout the study with tumor growth measured every 3 days.

[00467] Results: A dose response relationship is evident for the CWR22 xenograft tumor growth inhibition by deJ591-DM1. This is shown on a 7 day dosing interval study for 2 different doses of deJ591-DM1. At the higher dose, there is suppression of tumor growth with some reduction from the initial tumor volume. At the lower dose there is not the same reduction from initial tumor volume and there is a more rapid return to normal growth kinetics of approximately 10 days following the last dose administered compared to the higher dose.

Example 18 – Bone marrow involvement in advanced prostate cancer patients demonstrated on bone marrow biopsy

[00468] Bone marrow involvement in advanced prostate cancer is not routinely examined. We report on the results of bone marrow biopsies performed on advanced, hormone-refractory prostate cancer patients.

[00469] Screening diagnostic studies were performed on hormone-refractory prostate cancer patients to determine eligibility for two phase I radioimmunotherapy clinical trials. Studies included serologic testing, bone scan, CT scans of the head, chest, abdomen and pelvis, and a bone marrow biopsy taken from the iliac crest. A total of Thirty-nine patients have been screened thus far.

[00470] All patients had advanced disease as determined by bony or soft tissue metastases on imaging and/or three consecutive rises in serum PSA levels. All patients had received prior hormonal therapy, and the majority had received local therapy, including radical prostatectomy (N=15), radiotherapy (N=19), and/or chemotherapy (N=19). Sixteen patients (41%) had histologic evidence of metastatic prostate cancer on bone marrow biopsy. Of the thirty-nine screened patients, thirteen (33%) had significant bone marrow involvement (>10% involvement), making them ineligible for entry into these clinical trials.

[00471] Patients with bone marrow involvement had significantly higher serum alkaline phosphatase (ALP) levels (median 374 U/L vs. 96 U/L, $p<0.001$) and significantly lower serum hemoglobin (median 11.6 g/dL vs. 12.7 g/dL, $p=0.02$). There was no difference in serum hemoglobin between patients with prior chemotherapy and/or prior radiotherapy. When the ALP

was normal (<120 U/L) or elevated (>120 U/L,) 0% and 75% of patients, respectively, had metastatic prostate cancer on bone marrow biopsy ($p<0.0001$). Patients with bone scans indicating bony metastases in three or more different anatomic sites (spine, thorax, pelvis, appendicular or calvarium) vs. two or less sites, were more likely to have bone marrow involvement (54% vs. 9%, $p=0.01$). Age, initial Gleason sum, serum PSA, PSA doubling time, the presence of soft-tissue metastases, prior local treatment or chemotherapy, or other hematologic parameters (WBC, platelet count) were not significantly different between the two groups.

[00472] Bone marrow involvement in advanced prostate cancer patients is not routinely examined, but is present in a large minority of cases. Current clinical staging studies may significantly underestimate bone marrow involvement, although elevation of alkaline phosphatase or depressed serum hemoglobin may be correlative. This situation should be considered in clinical trials carrying potential hematologic toxicity.

Example 19: A Novel Sandwich Enzyme-Linked Immunoassay (ELISA) for Quantification of Prostate-Specific Membrane Antigen

[00473] Ideally, serum PSMA should be detectable with a simple, rapid, reproducible and quantitative ELISA assay. Our objective was to establish an ELISA assay to measure serum PSMA.

[00474] Ninety-six well plates were coated with an anti-PSMA antibody as a "capture" antibody. Dilutions of serum from males and females "spiked" with recombinant PSMA (rPSMA), semen, LNCaP lysates, as well as the standard (rPSMA range 1.6-1600 ng/ml) were then added to these wells. A non-competing biotinylated anti-PSMA antibody (that recognizes a different epitope on PSMA) was then added as the "detection" antibody. Avidin phosphatase followed by p-nitrophenyl phosphate (substrate) was added. Optical densities were then measured.

[00475] The standard curve for the assay was linear through a range of 5-1600 ng/ml (correlation coefficient > .99). Using this assay, PSMA was detected in LNCaP lysate, semen, and "spiked" serum.

Equivalents

[00476] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. A modified anti-prostate specific membrane antigen (PSMA) immunoglobulin, comprising the amino acid sequence shown as SEQ ID NO:22 or SEQ ID NO:50, or the light chain variable region amino acid sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

2. The modified PSMA immunoglobulin of claim 1, which is encoded by the nucleotide sequence shown as nucleic acid residues 261-581 of SEQ ID NO:25, SEQ ID NO:52, or the light chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

3. A modified anti-PSMA immunoglobulin, comprising a heavy chain variable region, comprising the amino acid sequence shown as SEQ ID NO:21 or SEQ ID NO:49, or the heavy chain variable region amino acid sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

4. The modified anti-PSMA immunoglobulin of claim 3, which is encoded by the nucleotide sequence shown as nucleic acid residues 261-605 of SEQ ID NO:23, SEQ ID NO:51, or the heavy chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

5. A modified anti-PSMA antibody, or an antigen-binding fragment thereof, comprising:

a light chain variable region comprising the amino acid sequence shown as SEQ ID NO:22 or SEQ ID NO:50, or the light chain variable region amino acid sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174; and

a heavy chain variable region comprising the amino acid sequence shown as SEQ ID NO:21 or SEQ ID NO:49, or the heavy chain variable region amino acid sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

6. The modified antibody, or fragment thereof, of claim 5, which comprises two heavy chains and two light chains.

7. The modified antibody, or fragment thereof, of claim 5, wherein the heavy chain constant region is of human isotype IgG1.

8. A pharmaceutical composition comprising the modified antibody, or fragment thereof, of claim 5, and a pharmaceutically acceptable carrier.

9. The modified antibody, or fragment thereof, of claim 5, further comprising a cytotoxic moiety or a detectable label.

10. The modified antibody, or fragment thereof, of claim 9, wherein the cytotoxic moiety is a cytotoxin, a therapeutic agent or a radioactive ion.

11. The modified antibody, or fragment thereof, of claim 10, wherein the radioactive ion is iodine (^{131}I), indium (^{111}In), yttrium (^{90}Y) or lutetium (^{177}Lu).

12. The modified antibody, or fragment thereof, of claim 10, wherein the cytotoxic moiety is maytansinoid.

13. The modified antibody, or fragment thereof, of claim 12, wherein the maytansinoid is maytansinol.

14. A nucleic acid sequence having a nucleotide sequence encoding a modified anti-PSMA immunoglobulin, comprising a light chain variable region comprising the nucleotide sequence shown as nucleic acid residues 261-581 of SEQ ID NO:25, SEQ ID NO:52, or the light chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

15. A nucleic acid sequence having a nucleotide sequence encoding a modified anti-PSMA immunoglobulin, comprising a heavy chain variable region comprising the nucleotide sequence shown as nucleic acid residues 261-605 of SEQ ID NO:23, SEQ ID NO:51, or the heavy chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

16. A first and second nucleic acid sequences having nucleotide sequences encoding light and heavy chain variable regions, respectively, of a modified anti-PSMA antibody or an antigen binding fragment thereof, wherein the light chain variable region comprises the nucleotide sequence shown as nucleic acid residues 261-581 of SEQ ID NO:25, SEQ ID NO:52, or the light chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174; and the heavy chain variable region comprises the nucleotide sequence shown as nucleic acid residues 261-605 of SEQ ID NO:2, SEQ ID NO:51, or the heavy chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

17. A method of producing a modified anti-PSMA antibody, or antigen binding fragment thereof, comprising:

providing a first nucleic acid encoding a light chain variable region comprising the nucleotide sequence shown as nucleic acid residues 261-581 of SEQ ID NO:25, SEQ ID NO:52, or the light chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174;

providing a second nucleic acid encoding a heavy chain variable region comprising the nucleotide sequence shown as nucleic acid residues 261-605 of SEQ ID NO:23, SEQ ID NO:51, or the heavy chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174; and

introducing said first and second nucleic acids into a host cell under conditions that allow expression and assembly of said light and heavy chain variable regions.

18. The method of claim 17, wherein the first and second nucleic acids are linked.

19. The method of claim 17, wherein the first and second nucleic acids are unlinked.

20. The method of claim 17, wherein the host cell is a eukaryotic or prokaryotic cell.

21. The method of claim 20, wherein the host cell is a mammalian cell.

22. The method of claim 21, wherein the mammalian cell is selected from the group consisting of a lymphocytic cell line, NS0 cell, CHO cell, COS cell, and a cell from a transgenic animal.

23. A method of ablating or killing a prostatic cell or a cancer cell, comprising contacting the cell, or a vascular endothelial cell proximate to cell, with the modified antibody, or fragment thereof of claim 5, such that the cell is ablated or killed upon binding of the antibody to the cell or the vascular endothelial cell proximate thereto.

24. A method of treating, or preventing, a prostatic or cancerous disorder, in a subject, comprising administering to the subject the modified antibody molecule of claim 5, in an amount effective to treat or prevent the disorder.

25. The method of claim 23 or 24, wherein the modified antibody, or fragment thereof, is associated with a cytotoxic agent or moiety.

26. The method of claim 25, wherein the cytotoxic agent or moiety is selected from the group consisting of an antimetabolite, an alkylating agent, cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines, and an anti-mitotic agent.

27. The method of claim 24, wherein modified antibody, or fragment thereof, is administered in combination with a cytotoxic agent.

28. The method of claim 25, wherein modified antibody, or fragment is coupled to a cytotoxic moiety.

29. The method of claim 28, wherein the cytotoxic moiety is a cytotoxin, a therapeutic agent or a radioactive isotope.

30. The method of claim 29, wherein the radioactive isotope is iodine (^{131}I), yttrium (^{90}Y) or lutetium (^{177}Lu).

31. The method of claim 29, wherein the cytotoxic moiety is maytansinoid.

32. The method of claim 31, wherein the maytansinoid is maytansinol.

33. The method of claim 31, wherein the maytansinoid is DM1.

34. The method of claims 23, wherein the cell is a malignant, normal, benign or hyperplastic, prostate epithelial cells.

35. The method of claim 23, wherein the cell is a renal, an urothelial, colon, rectal, lung, breast or liver, cancerous or metastatic cell.

36. The method of claim 24, wherein the disorder is selected from the group consisting of genitourinary inflammation, prostatitis, benign enlargement, prostatic cancer and testicular cancer.

37. The method of claim 24, wherein the disorder is selected from the group consisting of solid tumors, soft tissue tumors, and metastatic lesions.

38. The method of claim 37, wherein the solid tumors are malignancies selected from the group consisting of sarcomas, adenocarcinomas, and carcinomas.

39. The method of claim 37, wherein the disorder is a malignancy affecting lung, breast, lymphoid tissue, gastrointestinal tissue, colon, rectum, genitourinary tract, renal cells, urothelial cells, pharynx, bladder, liver, lung, small intestine and esophagus.

40. A method for detecting the presence of a PSMA protein, in a sample comprising prostatic or cancerous cells, *in vitro*, comprising: (i) contacting the sample or a reference sample with the modified anti-PSMA antibody, or fragment thereof, of claim 7, under conditions that allow interaction of the anti-PSMA antibody and the PSMA protein to occur; and (ii) detecting formation of a complex between the anti-PSMA antibody, and the sample or the reference sample, wherein a statistically significant change in the formation of the complex in the sample relative to a reference sample is indicative of the presence of PSMA in the sample.

41. A method for detecting the presence of PSMA *in vivo*, comprising: (i) administering to a subject the modified anti-PSMA antibody, or antigen binding fragment thereof, of claim 7, in detectably labeled form, under conditions that allow interaction of the modified anti-PSMA antibody, or fragment thereof, and the PSMA protein to occur; and (ii) detecting formation of a complex between the antibody or fragment and PSMA, wherein a statistically significant change in the formation of the complex in the subject relative to a reference is indicative of the presence of the PSMA.

42. A method of diagnosing or staging, a prostatic or cancerous disorder, comprising: (i) identifying a subject having, or at risk of having, the disorder; (ii) obtaining a sample of a tissue or cell affected with the disorder; (iii) contacting said sample or a control sample with the modified anti-PSMA antibody or fragment thereof of claim 7, under conditions that allow interaction of the binding agent and the PSMA protein to occur, and (iv) detecting formation of a complex, wherein a statistically significant increase in the formation of the complex between the antibody or fragment thereof with respect to the control sample, is indicative of the disorder or the stage of the disorder.

43. A method for *in vivo* imaging PSMA-expressing tumors comprising: (i) administering to a subject, or a a control subject a labeled modified anti-PSMA antibody or

fragment thereof of claim 7, under conditions that allow interaction of the modified anti-PSMA antibody or fragment thereof and the PSMA protein to occur; and (ii) detecting formation of a complex between the antibody or fragment and PSMA.

44. A method of treating pain experienced by a subject having or diagnosed with benign prostatic hyperplasia or prostate cancer, or non-prostate cancer, comprising: administering an anti-PSMA antibody to a subject in an amount sufficient to treat the pain associated with prostate disease or non-prostate cancer.

45. A method for treating metastatic lesions associated with prostate cancer in a subject, comprising administering to the subject the modified antibody molecule of claim 5, in an amount effective to treat or prevent the disorder.

Amino Acid Sequence of Murine J591 Heavy Chain (CDRs are marked, numbering as Kabat)

```
EVQLQQSGPELKKPGTSVRISCKTS|GYTFTEYTIH|WVKQSHGKS
1         10         20          30          40
                                 CDR1

LEWIG|NINPNNGGTTYNQKFED|KATLTVDKSSSTAYMELRSLTS
      50          60          70          80
             CDR2

EDSAVYYCAA|GWNFDY|WGQGTTLTVSS
     90        100         110
            CDR3
```

FIG. 1A

Amino Acid Sequence of Murine J591 Light Chain (CDRs are marked, numbering as Kabat)

```
DIVMTQSHKFMSTSVGDRVSIIC|KASQDVGTAVD|WYQQKPGQSP
1         10         20          30          40
                               CDR1

KLLIY|WASTRHT|GVPDRFTGSGSGTDFTLTITNVQSEDLADYFC
      50          60          70          80
        CDR2

|QQYNSYPLT|FGAGTMLDLK
  90         100
   CDR3
```

FIG. 1B

Amino Acid Sequence of DeImmunised J591 Heavy Chain (CDRs are marked, numbering as Kabat)

```
EVQLVQSGPEVKKPGATVKISCKTS|GYTFTEYTIH|WVKQAPGKG
1         10        20         30        40
                                CDR1

LEWIG|NINPNNGGTTYNQKFED|KATLTVDKSTDTAYMELSSLRS
      50         60         70         80
           CDR2

EDTAVYYCAA|GWNFDY|WGQGTLLTVSS
     90       100        110
           CDR3
```

FIG. 2A

Amino Acid Sequence of DeImmunised J591 Light Chain (CDRs are marked, numbering as Kabat)

```
DIQMTQSPSSLSTSVGDRVTLTC|KASQDVGTAVD|WYQQKPGPSP
1         10        20         30        40
                           CDR1

KLLIY|WASTRHT|GIPSRFSGSGSGTDFTLTISSLQPEDFADYYC
     50         60         70         80
        CDR2

|QQYNSYPLT|FGPGTKVDIK
 90        100
   CDR3
```

FIG. 2B

Location of T cell epitopes in J591 VH

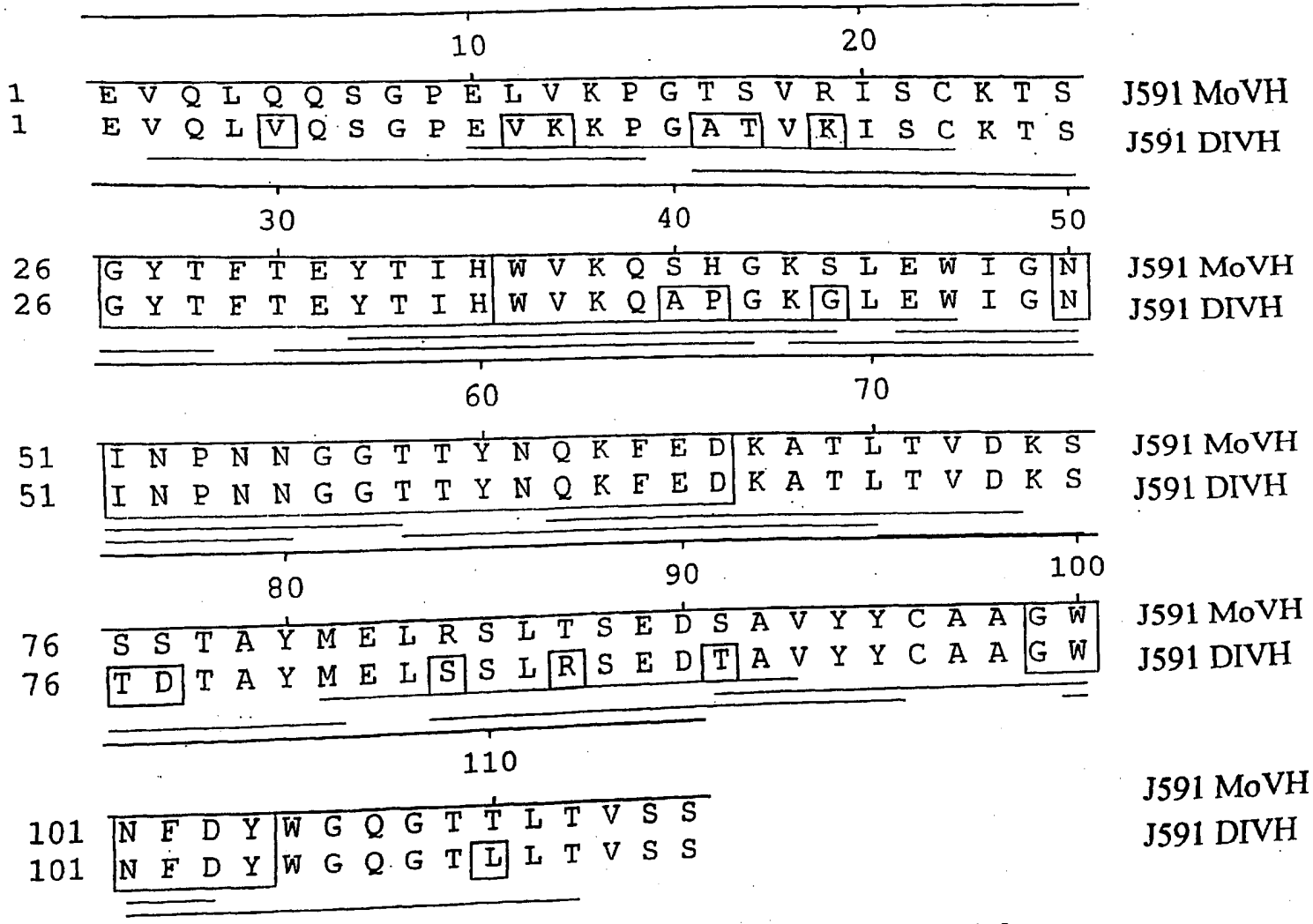


FIG. 3A

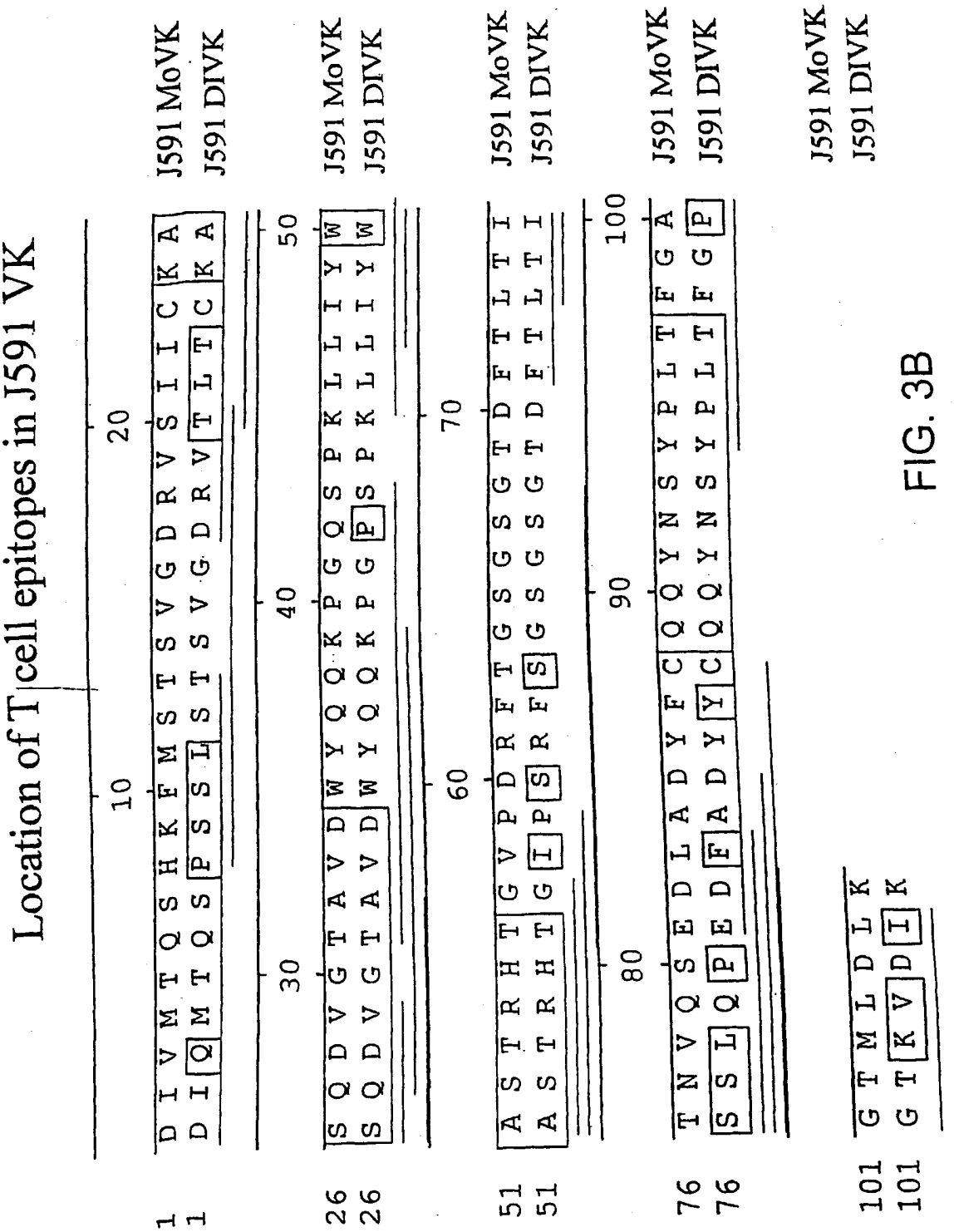

Location of T cell epitopes in J591 VK
J591 MoVK
J591 DIVK
1 D I V M T Q S H K F M S T S V G D R V S I I C K A
1 D I Q M T Q S P S S L S T S V G D R V T L T C K A
26 S Q D V G T A V D W Y Q Q K P G Q S P K L L I Y W
26 S Q D V G T A V D W Y Q Q K P G P S P K L L I Y W
51 A S T R H T G V P D R F T G S G S G T D F T L T I
51 A S T R H T G I P S R F S G S G S G T D F T L T I
76 T N V Q S E D L A D Y F C Q Q Y N S Y P L T F G A
76 S S L Q P E D F A D Y Y C Q Q Y N S Y P L T F G P
101 G T M L D L K
101 G T K V D I K

FIG. 3B

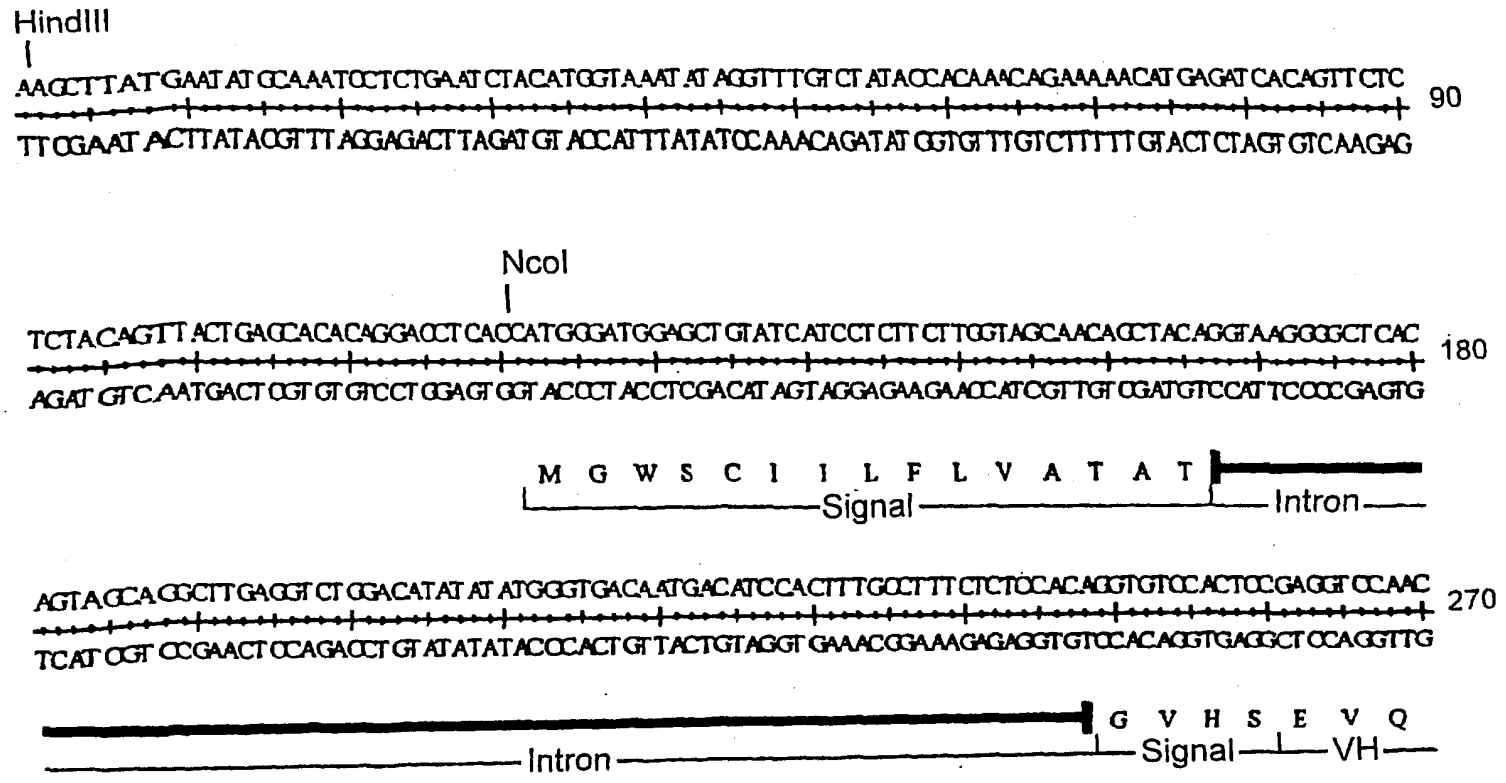

HindIII
AAGCTTATGAATATGCAAATCCTCTGAATCTACATGGTAAATATAGGTTTGTCTATACCACAAACAGAAAAACATGAGATCACAGTTCTC
90
TTCGAATACTTATACGTTTAGGAGACTTAGATGTACCATTTATATCCAAACAGATATCGTGTTTGTCTTTTTGTACTCTAGTGTCAAGAG
NcoI
TCTACAGTTACTGACCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACACCTACAGGTAAGGGCTCAC
180
AGATGTCAATGACTCGTGTGTCCTGGAGTGGTACCCTACCTCGACATAGTAGGAGAAGAACCATCGTTGTCGATGTCCATTCCCCGAGTG
M G W S C I I L F L V A T A T
Signal
Intron
AGTAGCACGCTTGAGGTCTCGACATATATATGGGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCGAGGTCCAAC
270
TCATCGTCCGAACTCCAGACCTGTATATATACCCACTGTTACTGTAGGTGAAACGGAAAGAGAGGTGTCCACAGGTGAGGCTCCAGGTTG
G V H S E V Q
Intron
Signal
VH

FIG. 4A-1

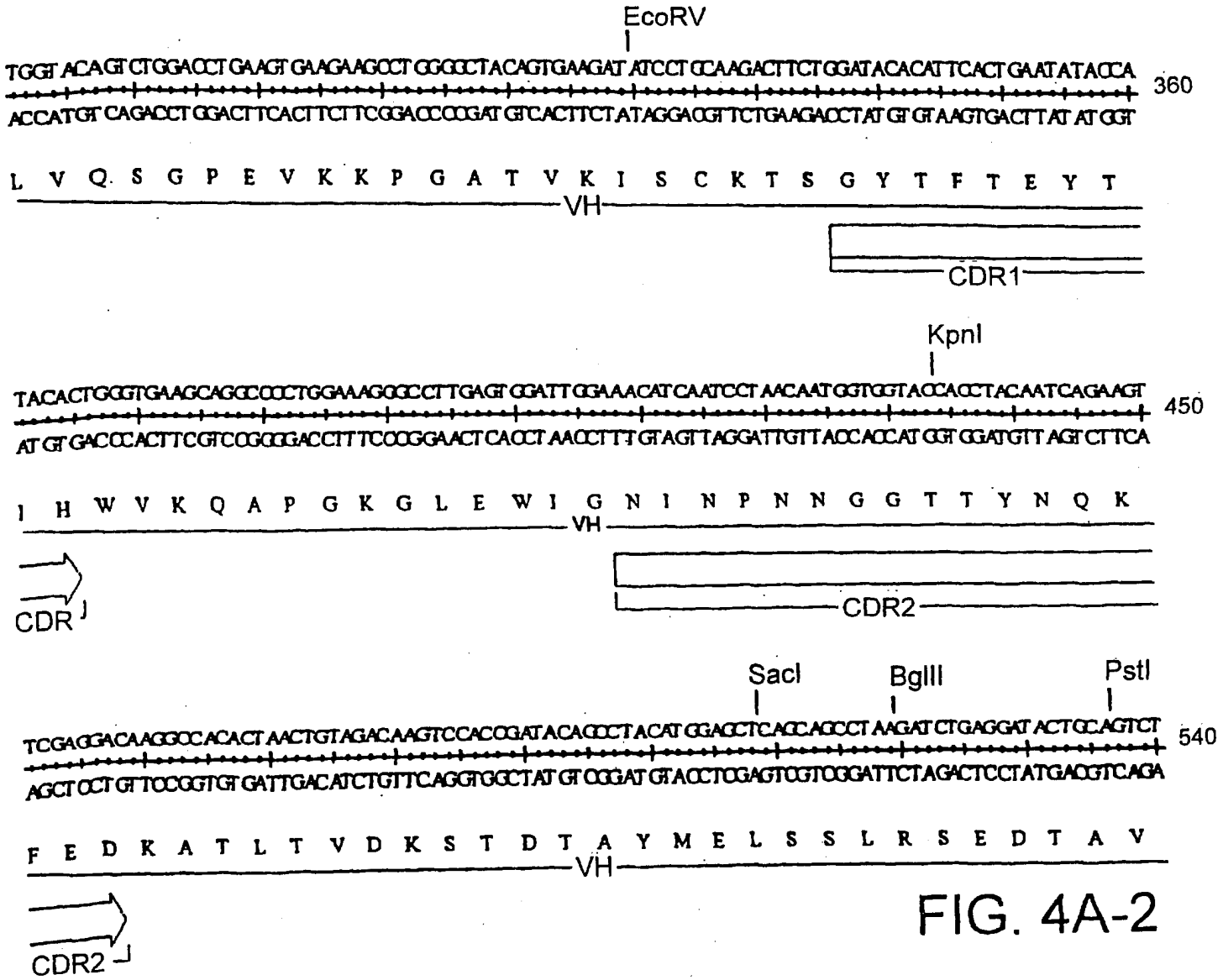

EcoRV
TGGT ACAGT CTGGACCT GAAGT GAAGAAGCCT GGGGCT ACAGTGAAGAT ATCCT GCAAGACTTCT GGATACACATTCACT GAATATACCA
360
ACCATGT CAGACCT GGACTTCACTTCTTCGGACCCCGAT GTCACTTCT ATAGGACGTTCTGAAGACCTAT GT GT AAGTGACTTAT AT GGT
L V Q S G P E V K K P G A T V K I S C K T S G Y T F T E Y T
VH
CDR1
KpnI
TACACTGGGTGAAGCAGGCCCCTGGAAAGGGCCTTGAGT GGATT GGAAACAT CAATCCT AACAAT GGTGGTACCACCTACAATCAGAAGT
450
AT GT GACCCACTTCGTCCGGGGACCTTTCCCGGAACT CACCT AACCTTT GTAGTTAGGATTGTTACCACCAT GGT GGATGTT AGT CTTCA
I H W V K Q A P G K G L E W I G N I N P N N G G T T Y N Q K
VH
CDR1
CDR2
SacI
BglII
PstI
TCGAGGACAAGGCCACACT AACTGTAGACAAGTCCACCGATACACCCTACAT GGAGCTCAGCAGCCT AAGAT CTGAGGAT ACTGCAGTCT
540
AGCT CCT GTTCCGGTGT GATTGACATCTGTTCAGGTGGCTAT GT CCGAT GTACCTCGAGTCGTCGGATTCTAGACTCCTATGACGTCAGA
F E D K A T L T V D K S T D T A Y M E L S S L R S E D T A V
VH
CDR2

FIG. 4A-2

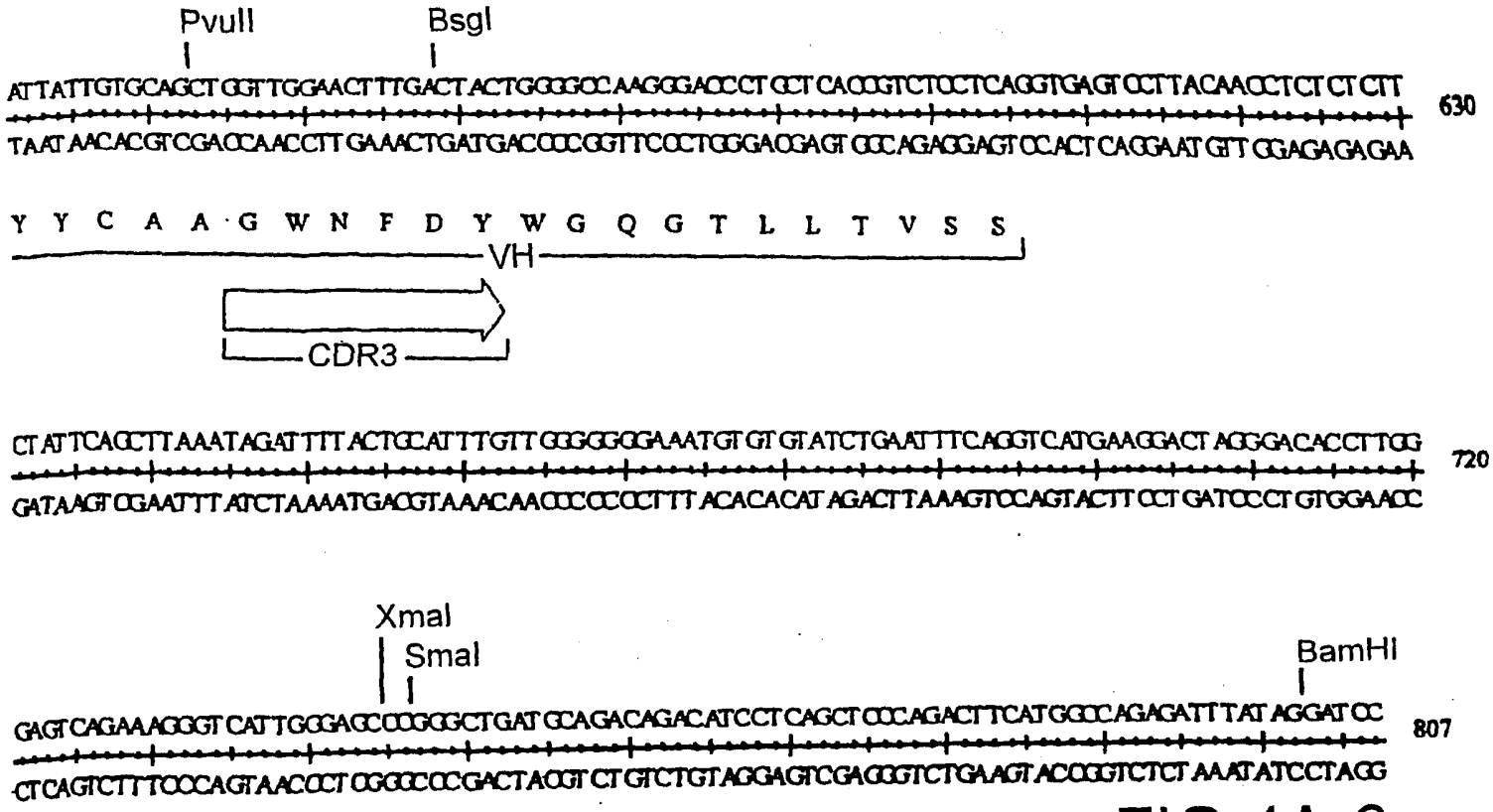

PvuII
BsgI
ATTATTGTGCAGCTGGTTGGAACTTTGACTACTGGGGCCAAGGGACCCTGCTCACCGTCTCCTCAGGTGAGTCCTTACAACCTCTCTCTT
630
TAATAACACGTCGACCAACCTTGAAACTGATGACCCCGGTTCCCTGGGACGAGTGGCCAGAGGAGTCCACTCAGGAATGTTGGAGAGAAGAA
Y Y C A A G W N F D Y W G Q G T L L T V S S
VH
CDR3
CTATTCAGCTTAAATAGATTTTACTGCATTTGTTGGGGGGGAAATGTGTGTATCTGAATTTCAGGTCATGAAGGACTAGGACACCTTGG
720
GATAAGTCGAATTTATCTAAAATGACGTAAACAACCCCCCCTTTACACACATAGACTTAAAGTCCAGTACTTCCTGATCCCTGTGGAACC
XmaI
SmaI
BamHI
GAGTCAGAAAGGGTCATTGGGAGCCCGGGCTGATGCAGACAGACATCCTCAGCTCCCAGACTTCATGGCCAGAGATTTTATAGGATCC
807
CTCAGTCTTTCCCAGTAACCCTCGGGCCCGACTACGTCTGTCTGTAGGAGTCGAGGGTCTGAAGTACCGGTCTCTAAATATCCTAGG

FIG 4A-3

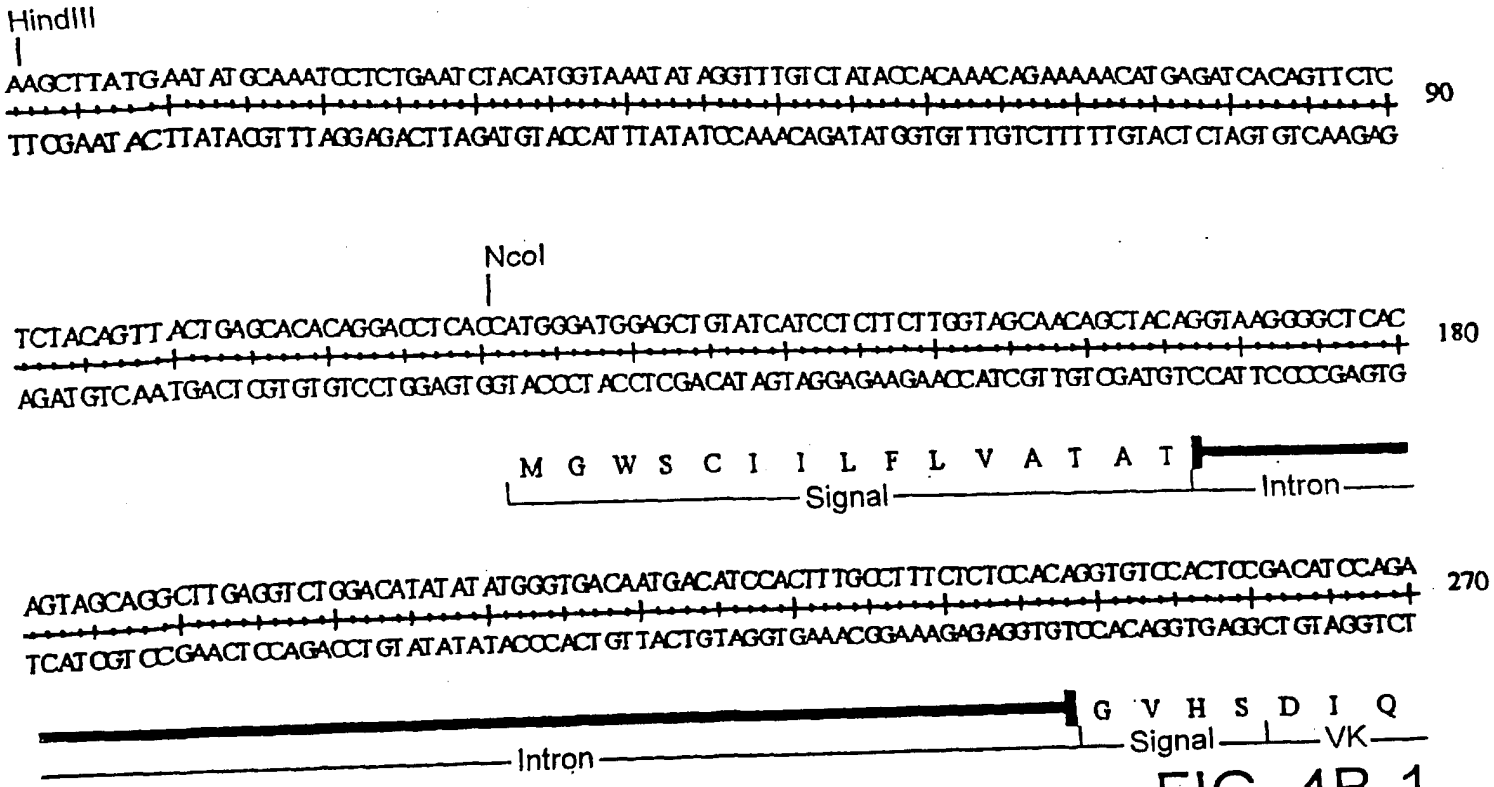

HindIII
AAGCTTATGAATATGCAAATCCTCTGAATCTACATGGTAAATATAGGTTTGTCTATACCACAAACAGAAAAACATGAGATCACAGTTCTC
90
TTCGAATACTTATACGTTTAGGAGACTTAGATGTACCATTTATATCCAAACAGATATGGTGTTTGTCTTTTTGTACTCTAGTGTCAAGAG
NcoI
TCTACAGTTACTGAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTAAGGGGCTCAC
180
AGATGTCAATGACTCGTGTGTCCTGGAGTGGTACCCTACCTCGACATAGTAGGAGAAGAACCATCGTTGTCGATGTCCATTCCCCGAGTG
M G W S C I I L F L V A T A T
Signal
Intron
AGTAGCAGGCTTGAGGTCTGGACATATATATGGGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCGACATCCAGA
270
TCATCGTCCGAACTCCAGACCTGTATATATACCCACTGTTACTGTAGGTGAAACGGAAAGAGAGGTGTCCACAGGTGAGGCTGTAGGTCT
G V H S D I Q
Signal
VK
Intron

FIG. 4B-1

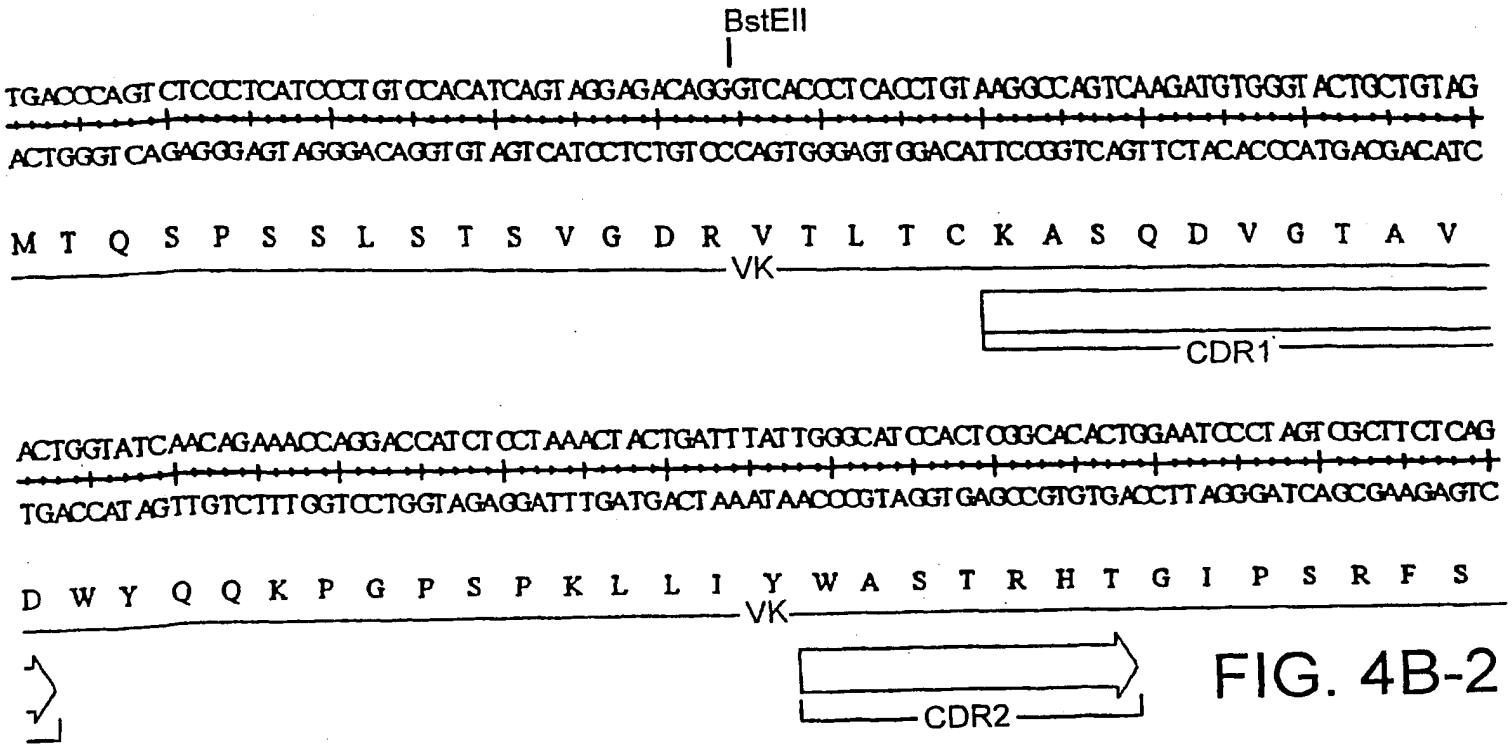

BstEII
TGACCCAGT CTCCCTCATCCCT GT CCACATCAGT AGGAGACAGGGTCACCCT CACCT GT AAGGCCAGTCAAGATGTGGGT ACTGCTGT AG
360
ACTGGGT CAGAGGGAGT AGGGACAGGT GT AGT CATCCTCTGT CCCAGTGGGAGT GGACATTCCGGTCAGTTCTACACCCATGACGACATC
M T Q S P S S L S T S V G D R V T L T C K A S Q D V G T A V
VK
CDR1
ACTGGTATCAACAGAAACCAGGACCATCT CCT AAACT ACTGATTTATTGGGCAT CCACT CGGCACACTGGAATCCCT AGT CGCTTCTCAG
450
TGACCAT AGTTGTCTTT GGTCCTGGTAGAGGATTTGATGACT AAATAACCCGTAGGTGAGCCGTGTGACCTT AGGGATCAGCGAAGAGTC
D W Y Q Q K P G P S P K L L I Y W A S T R H T G I P S R F S
VK
CDR2

FIG. 4B-2

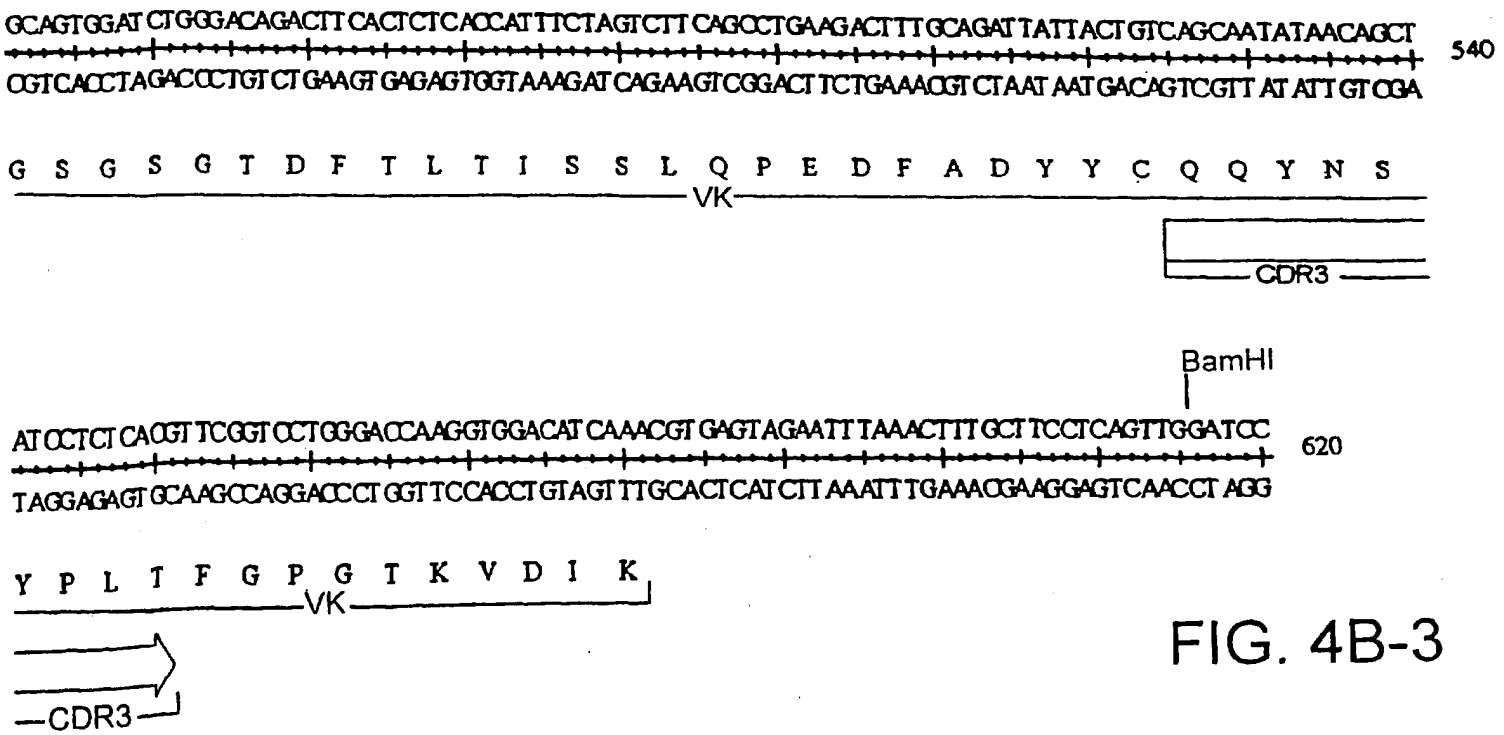

GCAGTGGAT CTGGGACAGACTT CACTCTC ACCATTTCTAGTCTT CAGCCTGAAGACTTTGCAGATTATTACTGTCAGCAATATAACAGCT
CGTCACCTAGACCCTGTCTGAAGTGAGAGTGGTAAAGATCAGAAGTCGGACTTCTGAAACGTCTAATAATGACAGTCGTTATATTGTCGA
540
G S G S G T D F T L T I S S L Q P E D F A D Y Y C Q Q Y N S
VK
CDR3
BamHI
ATCCTCTCACGTTCGGTCCTGGGACCAAGGTGGACATCAAACGTGAGTAGAATTTAAACTTTGCTTCCTCAGTTGGATCC
TAGGAGAGTGCAAGCCAGGACCCTGGTTCCACCTGTAGTTTGCACTCATCTTAAATTTGAAACGAAGGAGTCAACCTAGG
620
Y P L T F G P G T K V D I K
VK
CDR3

FIG. 4B-3

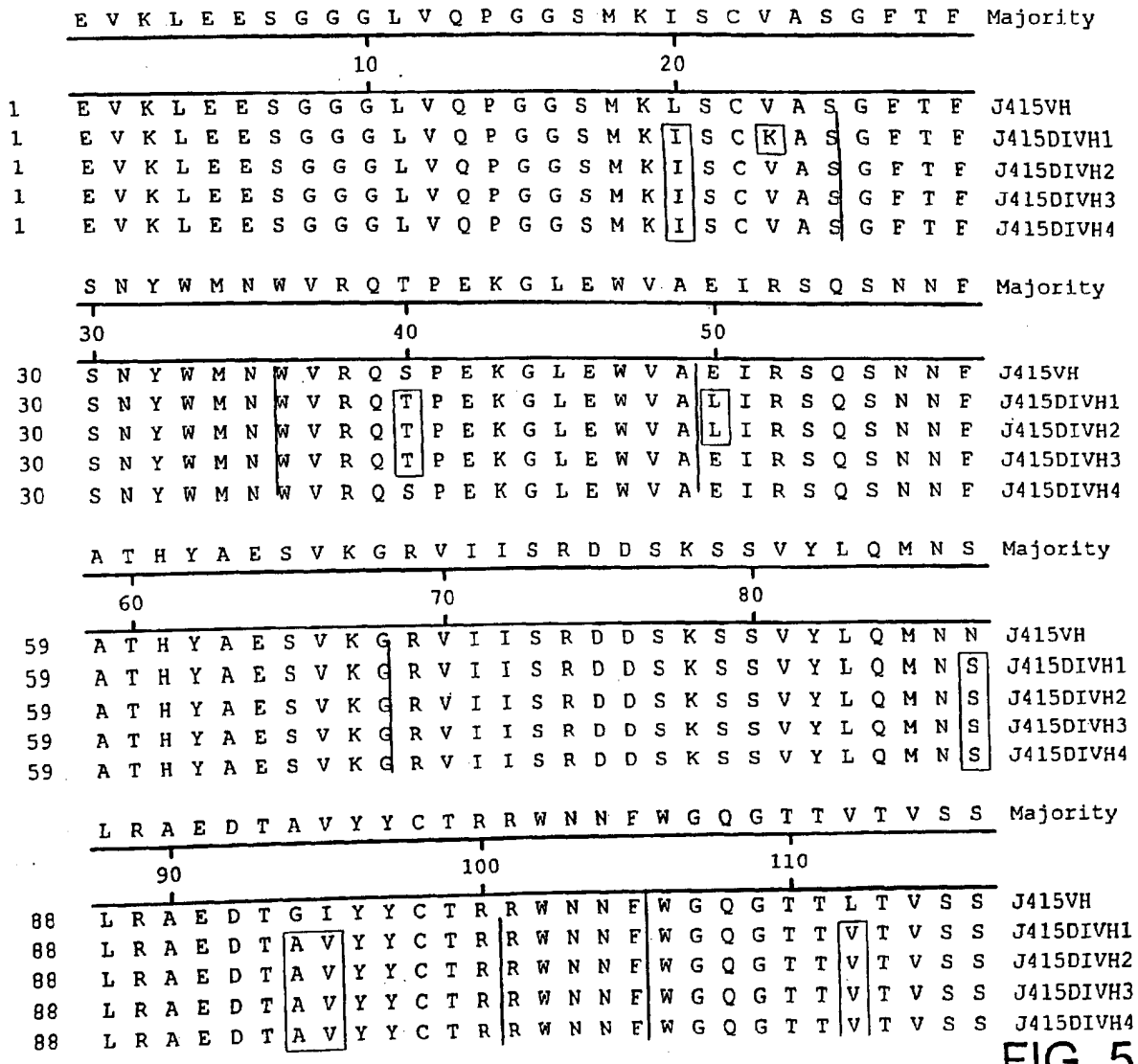

E V K L E E S G G G L V Q P G G S M K I S C V A S G F T F Majority
10 20
1 E V K L E E S G G G L V Q P G G S M K L S C V A S G F T F J415VH
1 E V K L E E S G G G L V Q P G G S M K I S C K A S G F T F J415DIVH1
1 E V K L E E S G G G L V Q P G G S M K I S C V A S G F T F J415DIVH2
1 E V K L E E S G G G L V Q P G G S M K I S C V A S G F T F J415DIVH3
1 E V K L E E S G G G L V Q P G G S M K I S C V A S G F T F J415DIVH4
S N Y W M N W V R Q T P E K G L E W V A E I R S Q S N N F Majority
30 40 50
30 S N Y W M N W V R Q S P E K G L E W V A E I R S Q S N N F J415VH
30 S N Y W M N W V R Q T P E K G L E W V A L I R S Q S N N F J415DIVH1
30 S N Y W M N W V R Q T P E K G L E W V A L I R S Q S N N F J415DIVH2
30 S N Y W M N W V R Q T P E K G L E W V A E I R S Q S N N F J415DIVH3
30 S N Y W M N W V R Q S P E K G L E W V A E I R S Q S N N F J415DIVH4
A T H Y A E S V K G R V I I S R D D S K S S V Y L Q M N S Majority
60 70 80
59 A T H Y A E S V K G R V I I S R D D S K S S V Y L Q M N N J415VH
59 A T H Y A E S V K G R V I I S R D D S K S S V Y L Q M N S J415DIVH1
59 A T H Y A E S V K G R V I I S R D D S K S S V Y L Q M N S J415DIVH2
59 A T H Y A E S V K G R V I I S R D D S K S S V Y L Q M N S J415DIVH3
59 A T H Y A E S V K G R V I I S R D D S K S S V Y L Q M N S J415DIVH4
L R A E D T A V Y Y C T R R W N N F W G Q G T T V T V S S Majority
90 100 110
88 L R A E D T G I Y Y C T R R W N N F W G Q G T T L T V S S J415VH
88 L R A E D T A V Y Y C T R R W N N F W G Q G T T V T V S S J415DIVH1
88 L R A E D T A V Y Y C T R R W N N F W G Q G T T V T V S S J415DIVH2
88 L R A E D T A V Y Y C T R R W N N F W G Q G T T V T V S S J415DIVH3
88 L R A E D T A V Y Y C T R R W N N F W G Q G T T V T V S S J415DIVH4

FIG. 5

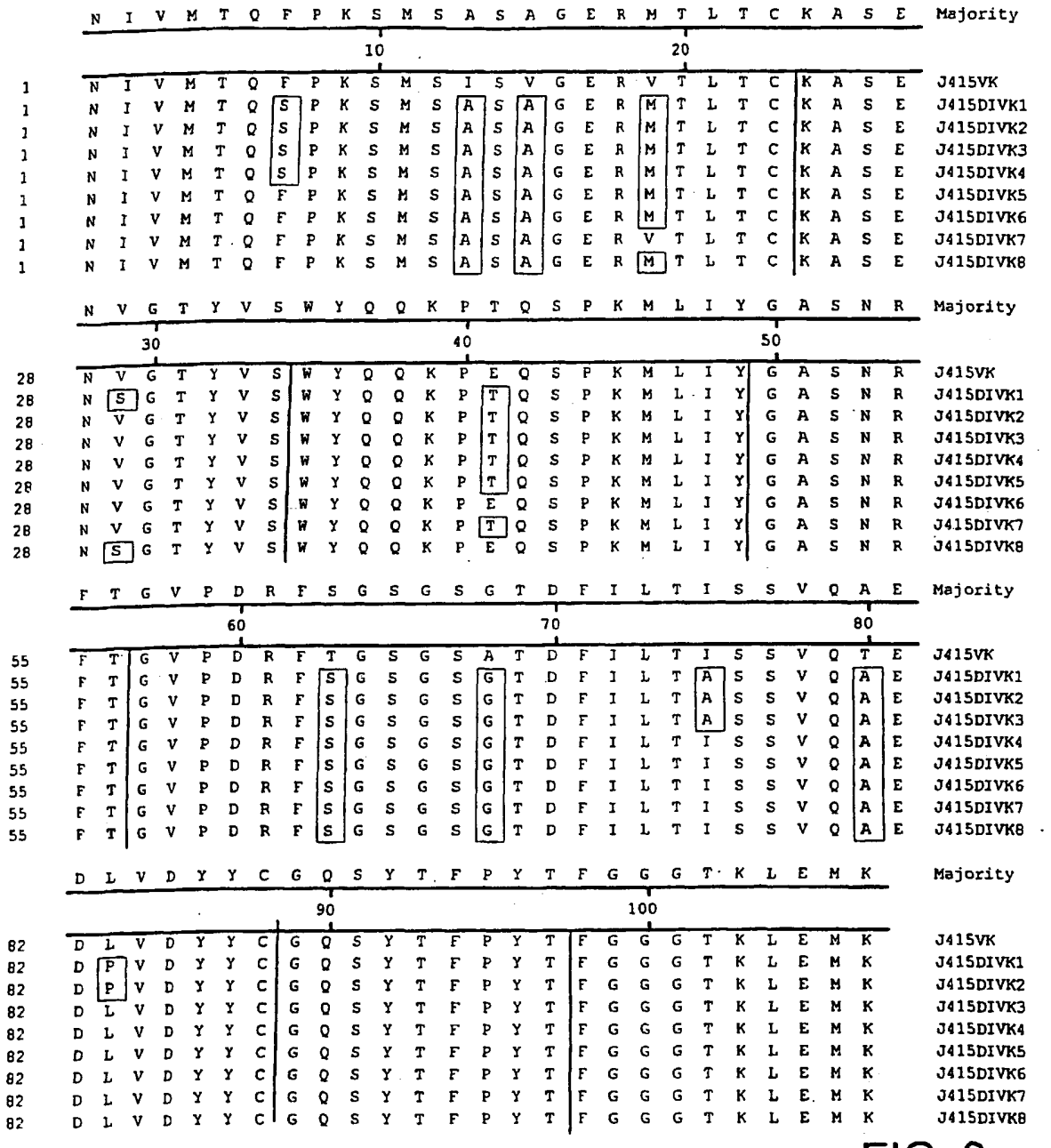

Majority N I V M T Q F P K S M S A S A G E R M T L T C K A S E
10
20
1 N I V M T Q F P K S M S I S V G E R V T L T C K A S E J415VK
1 N I V M T Q S P K S M S A S A G E R M T L T C K A S E J415DIVK1
1 N I V M T Q S P K S M S A S A G E R M T L T C K A S E J415DIVK2
1 N I V M T Q S P K S M S A S A G E R M T L T C K A S E J415DIVK3
1 N I V M T Q S P K S M S A S A G E R M T L T C K A S E J415DIVK4
1 N I V M T Q F P K S M S A S A G E R M T L T C K A S E J415DIVK5
1 N I V M T Q F P K S M S A S A G E R M T L T C K A S E J415DIVK6
1 N I V M T Q F P K S M S A S A G E R V T L T C K A S E J415DIVK7
1 N I V M T Q F P K S M S A S A G E R M T L T C K A S E J415DIVK8
Majority N V G T Y V S W Y Q Q K P T Q S P K M L I Y G A S N R
30
40
50
28 N V G T Y V S W Y Q Q K P E Q S P K M L I Y G A S N R J415VK
28 N S G T Y V S W Y Q Q K P T Q S P K M L I Y G A S N R J415DIVK1
28 N V G T Y V S W Y Q Q K P T Q S P K M L I Y G A S N R J415DIVK2
28 N V G T Y V S W Y Q Q K P T Q S P K M L I Y G A S N R J415DIVK3
28 N V G T Y V S W Y Q Q K P T Q S P K M L I Y G A S N R J415DIVK4
28 N V G T Y V S W Y Q Q K P T Q S P K M L I Y G A S N R J415DIVK5
28 N V G T Y V S W Y Q Q K P E Q S P K M L I Y G A S N R J415DIVK6
28 N V G T Y V S W Y Q Q K P T Q S P K M L I Y G A S N R J415DIVK7
28 N S G T Y V S W Y Q Q K P E Q S P K M L I Y G A S N R J415DIVK8
Majority F T G V P D R F S G S G S G T D F I L T I S S V Q A E
60
70
80
55 F T G V P D R F T G S G S A T D F I L T I S S V Q T E J415VK
55 F T G V P D R F S G S G S G T D F I L T A S S V Q A E J415DIVK1
55 F T G V P D R F S G S G S G T D F I L T A S S V Q A E J415DIVK2
55 F T G V P D R F S G S G S G T D F I L T A S S V Q A E J415DIVK3
55 F T G V P D R F S G S G S G T D F I L T I S S V Q A E J415DIVK4
55 F T G V P D R F S G S G S G T D F I L T I S S V Q A E J415DIVK5
55 F T G V P D R F S G S G S G T D F I L T I S S V Q A E J415DIVK6
55 F T G V P D R F S G S G S G T D F I L T I S S V Q A E J415DIVK7
55 F T G V P D R F S G S G S G T D F I L T I S S V Q A E J415DIVK8
Majority D L V D Y Y C G Q S Y T F P Y T F G G G T K L E M K
90
100
82 D L V D Y Y C G Q S Y T F P Y T F G G G T K L E M K J415VK
82 D P V D Y Y C G Q S Y T F P Y T F G G G T K L E M K J415DIVK1
82 D P V D Y Y C G Q S Y T F P Y T F G G G T K L E M K J415DIVK2
82 D L V D Y Y C G Q S Y T F P Y T F G G G T K L E M K J415DIVK3
82 D L V D Y Y C G Q S Y T F P Y T F G G G T K L E M K J415DIVK4
82 D L V D Y Y C G Q S Y T F P Y T F G G G T K L E M K J415DIVK5
82 D L V D Y Y C G Q S Y T F P Y T F G G G T K L E M K J415DIVK6
82 D L V D Y Y C G Q S Y T F P Y T F G G G T K L E M K J415DIVK7
82 D L V D Y Y C G Q S Y T F P Y T F G G G T K L E M K J415DIVK8

FIG. 6

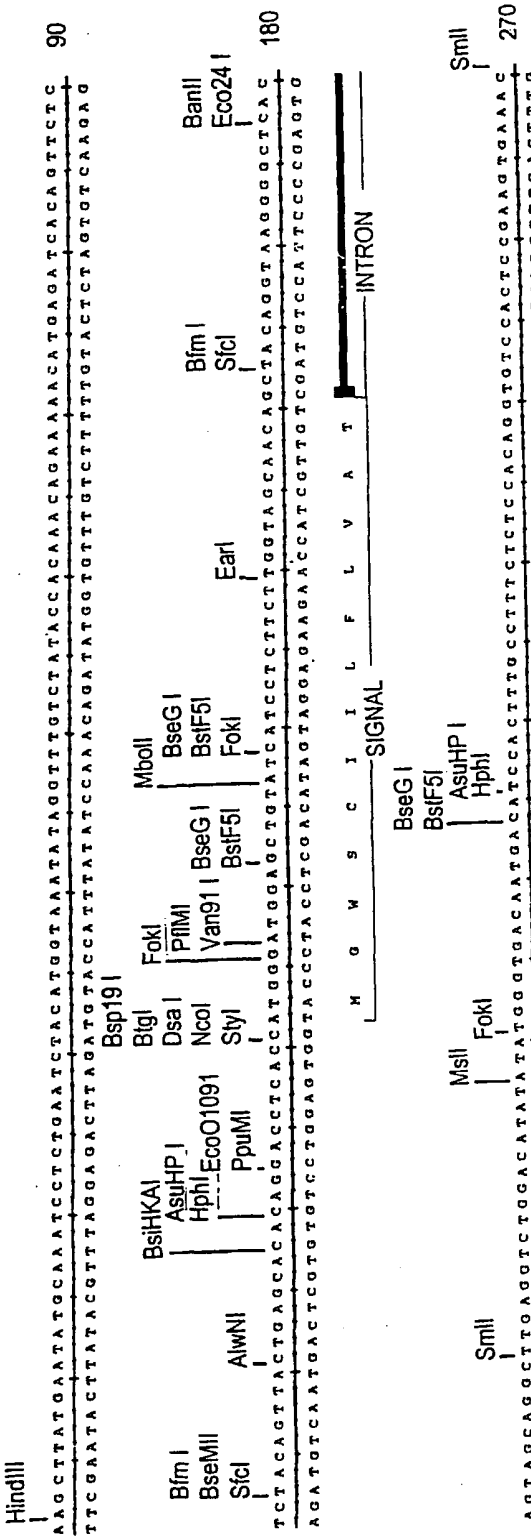

HindIII
Bsp19 I
BsiHKAI
AsuHP I
HphI
EcoO1091
PpuMI
AlwNI
BtgI
Dsa I
NcoI
StyI
FokI
PflMI
Van91 I
BseG I
BstF5I
MboII
BseG I
BstF5I
FokI
EarI
Bfm I
SfcI
BanII
Eco24 I
SIGNAL
INTRON
BseG I
BstF5I
AsuHP I
HphI
SmlI
MslI
FokI
SmlI
INTRON
SIGNAL
J415DIVH1

FIG. 7A-1

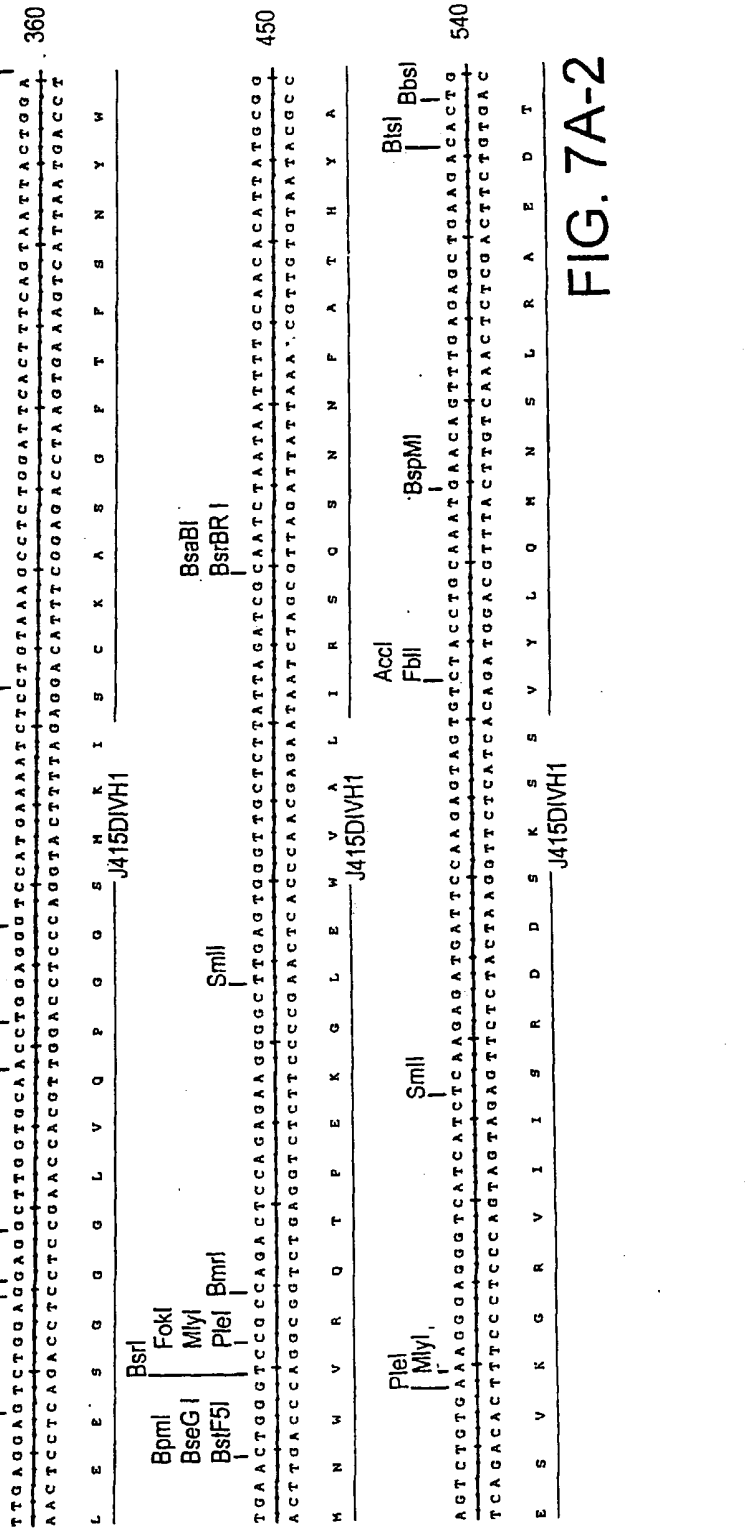

BsaXI
PleI
MlyI
BseRI
BseRI
BpmI
BsaXI
BpmI
BsrI
EcII
360
L E E S G G G L V Q P G G S M K I S C K A S G F T F S N Y W
J415DIVH1
BsrI
FokI
MlyI
PleI
BmrI
SmlI
BsaBI
BsrBR I
BpmI
BseG I
BstF5I
450
M N W V R Q T P E K G L E W V A L I R S Q S N N F A T H Y A
J415DIVH1
AccI
FblI
BspMI
PleI
MlyI
SmlI
BtsI
BbsI
540
E S V K G R V I I S R D D S K S S V Y L Q M N S L R A E D T
J415DIVH1

FIG. 7A-2

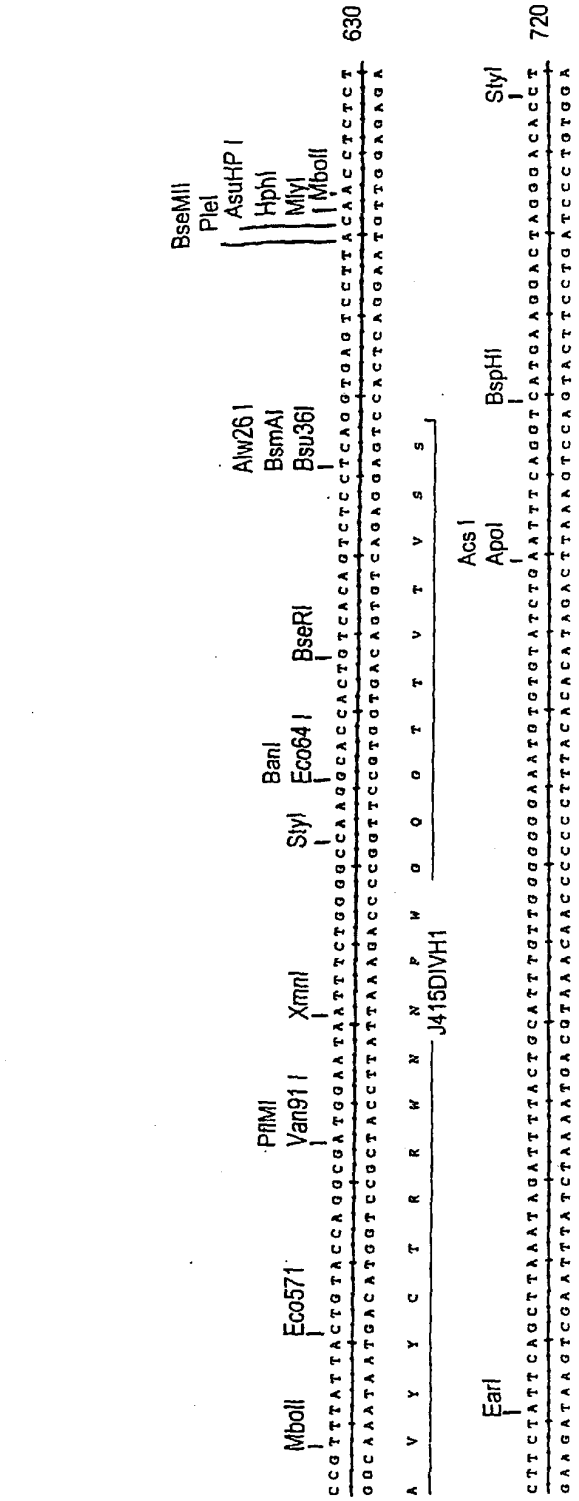

MboII
Eco571
PflMI
Van91 I
XmnI
StyI
BanI
Eco64 I
BseRI
Alw26 I
BsmAI
Bsu36I
BseMII
PleI
AsuHP I
HphI
MlyI
MboII
J415DIVH1
EarI
AcsI
ApoI
BspHI
StyI
SfaNI
Cfr9 I
Eco88 I
PspA I
XmaI
BanII
AvaI
Eco24 I
SmaI
SrfI
FokI
BseG I
BsiF5I
BbvCI
Bpu10I
BseMII
EaeI
Bal I
MscI
AlwI
BamHI
BstYI
Xho II
BsmFI
PleI
MlyI

FIG. 7A-3

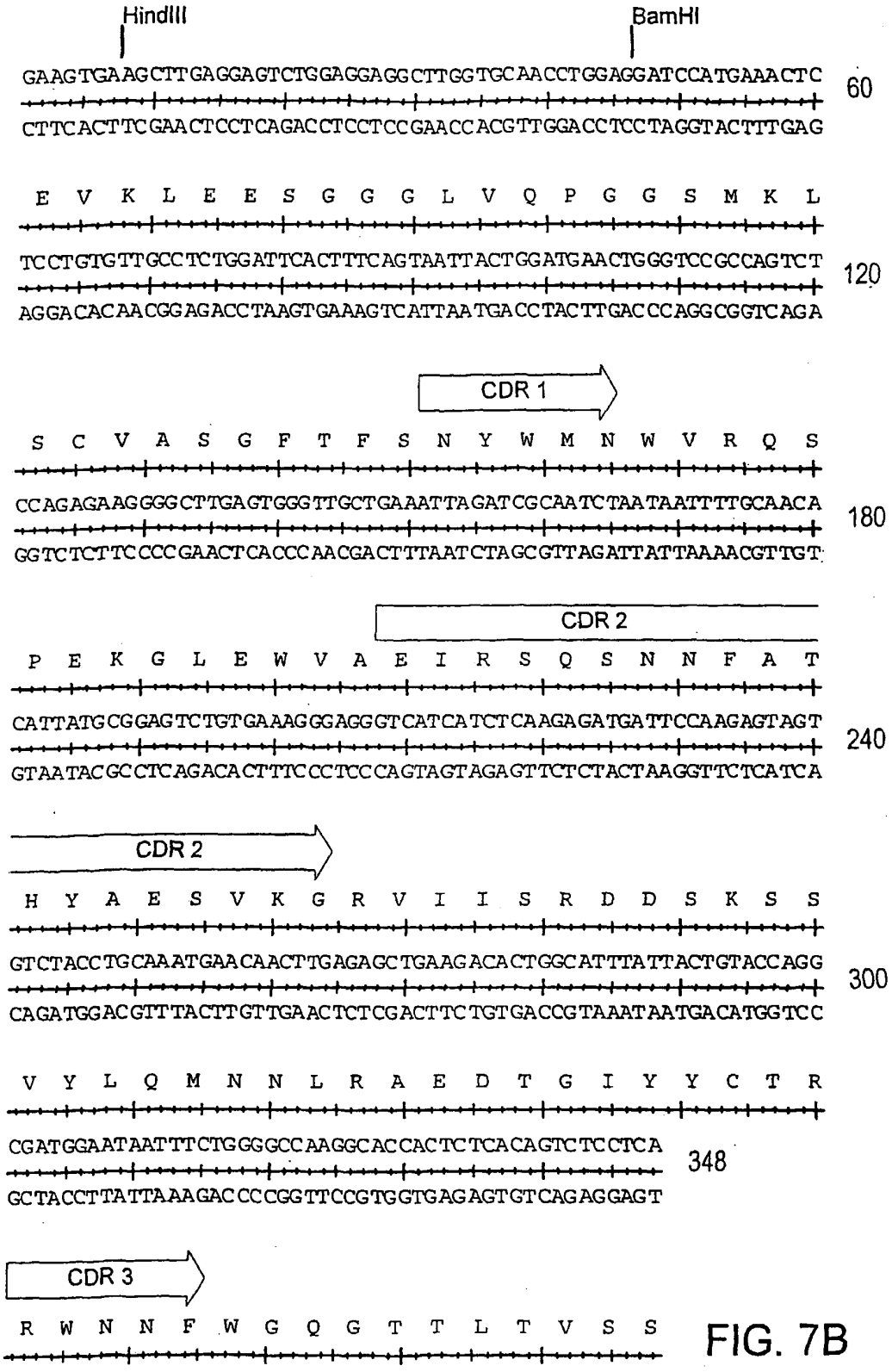

HindIII
BamHI
GAAGTGAAGCTTGAGGAGTCTGGAGGAGGCTTGGTGCAACCTGGAGGATCCATGAAACTC
60
CTTCACTTCGAACTCCTCAGACCTCCTCCGAACCACGTTGGACCTCCTAGGTACTTTGAG
E V K L E E S G G G L V Q P G G S M K L
TCCTGTGTTGCCTCTGGATTCACTTTCAGTAATTACTGGATGAACTGGGTCCGCCAGTCT
120
AGGACACAACGGAGACCTAAGTGAAAGTCATTAATGACCTACTTGACCCAGGCGGTCAGA
CDR 1
S C V A S G F T F S N Y W M N W V R Q S
CCAGAGAAGGGGCTTGAGTGGGTTGCTGAAATTAGATCGCAATCTAATAATTTTGCAACA
180
GGTCTCTTCCCCGAACTCACCCAACGACTTTAATCTAGCGTTAGATTATTAAAACGTTGT
CDR 2
P E K G L E W V A E I R S Q S N N F A T
CATTATGCGGAGTCTGTGAAAGGAGGGTCATCATCTCAAGAGATGATTCCAAGAGTAGT
240
GTAATACGCCTCAGACACTTTCCCTCCCAGTAGTAGAGTTCTCTACTAAGGTTCTCATCA
CDR 2
H Y A E S V K G R V I I S R D D S K S S
GTCTACCTGCAAATGAACAACTTGAGAGCTGAAGACACTGGCATTTATTACTGTACCAGG
300
CAGATGGACGTTTACTTGTTGAACTCTCGACTTCTGTGACCGTAAATAATGACATGGTCC
V Y L Q M N N L R A E D T G I Y Y C T R
CGATGGAATAATTTCTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
348
GCTACCTTATTAAAGACCCCGGTTCCGTGGTGAGAGTGTCAGAGGAGT
CDR 3
R W N N F W G Q G T T L T V S S

FIG. 7B

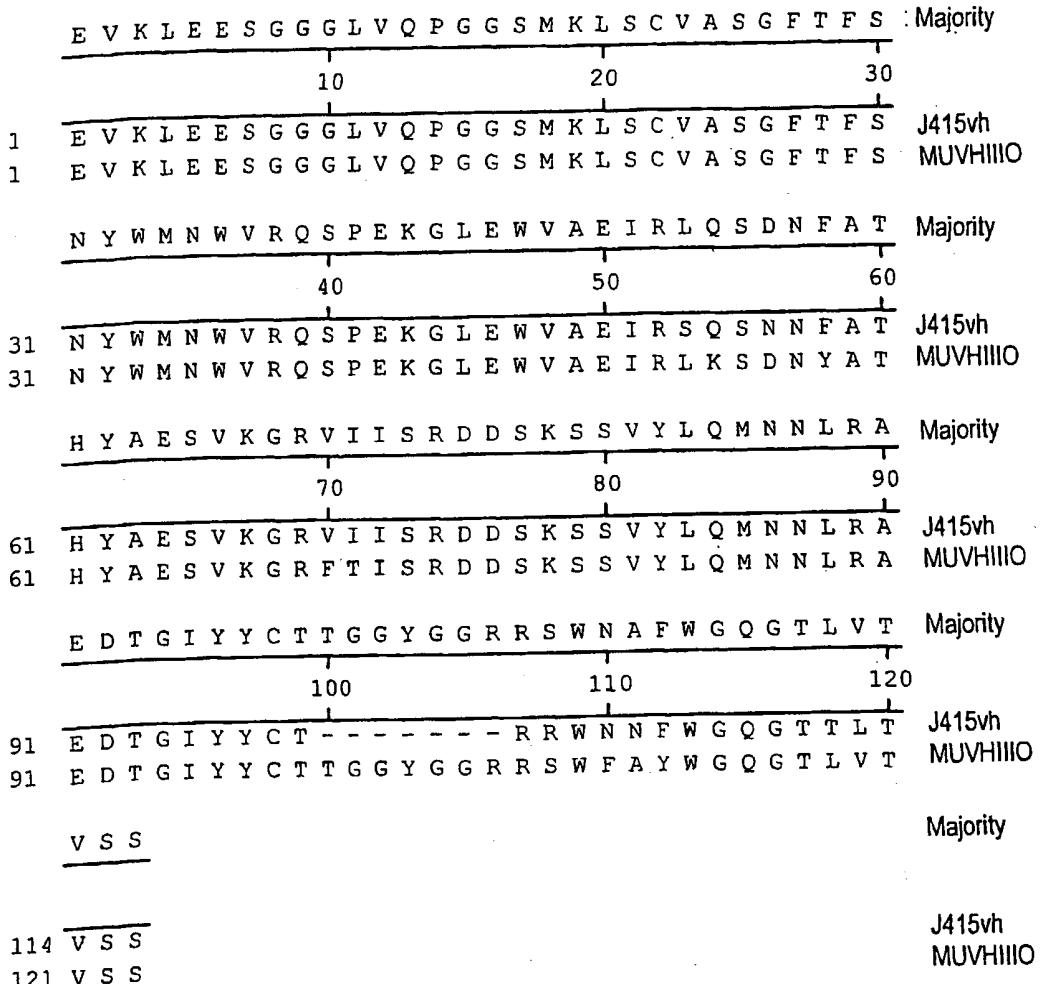

E V K L E E S G G G L V Q P G G S M K L S C V A S G F T F S : Majority
10 20 30
1 E V K L E E S G G G L V Q P G G S M K L S C V A S G F T F S J415vh
1 E V K L E E S G G G L V Q P G G S M K L S C V A S G F T F S MUVHIIIO
N Y W M N W V R Q S P E K G L E W V A E I R L Q S D N F A T Majority
40 50 60
31 N Y W M N W V R Q S P E K G L E W V A E I R S Q S N N F A T J415vh
31 N Y W M N W V R Q S P E K G L E W V A E I R L K S D N Y A T MUVHIIIO
H Y A E S V K G R V I I S R D D S K S S V Y L Q M N N L R A Majority
70 80 90
61 H Y A E S V K G R V I I S R D D S K S S V Y L Q M N N L R A J415vh
61 H Y A E S V K G R F T I S R D D S K S S V Y L Q M N N L R A MUVHIIIO
E D T G I Y Y C T T G G Y G G R R S W N A F W G Q G T L V T Majority
100 110 120
91 E D T G I Y Y C T - - - - - - - R R W N N F W G Q G T T L T J415vh
91 E D T G I Y Y C T T G G Y G G R R S W F A Y W G Q G T L V T MUVHIIIO
V S S Majority
114 V S S J415vh
121 V S S MUVHIIIO

FIG. 7C

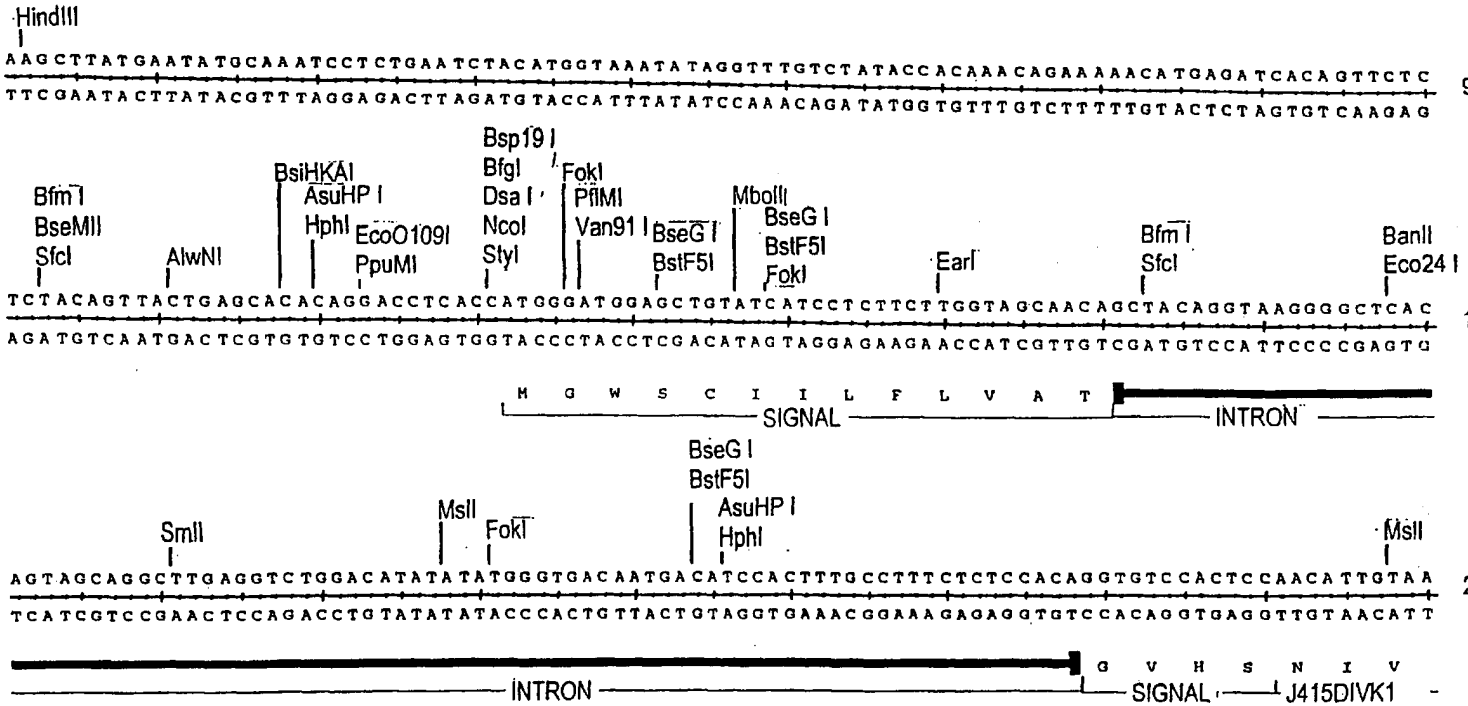

HindIII
AAGCTTATGAATATGCAAATCCTCTGAATCTACATGGTAAATATAGGTTTGTCTATACCACAAACAGAAAAACATGAGATCACAGTTCTC
TTCGAATACTTATACGTTTAGGAGACTTAGATGTACCATTTATATCCAAACAGATATGGTGTTTGTCTTTTTGTACTCTAGTGTCAAGAG
90
Bfm I
BseMII
SfcI
AlwNI
BsiHKAI
AsuHP I
HphI
EcoO109I
PpuMI
Bsp19 I
Bfgl
Dsa I
NcoI
StyI
FokI
PflMI
Van91 I
BseG I
BstF5I
MboII
BseG I
BstF5I
FokI
EarI
Bfm I
SfcI
BanII
Eco24 I
TCTACAGTTACTGAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTAAGGGGCTCAC
AGATGTCAATGACTCGTGTGTCCTGGAGTGGTACCCTACCTCGACATAGTAGGAGAAGAACCATCGTTGTCGATGTCCATTCCCCGAGTG
180
M G W S C I I L F L V A T
SIGNAL
INTRON
BseG I
BstF5I
AsuHP I
HphI
SmII
MslI
FokI
MslI
AGTAGCAGGCTTGAGGTCTGGACATATATATGGGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCAACATTGTAA
TCATCGTCCGAACTCCAGACCTGTATATATACCCACTGTTACTGTAGGTGAAACGGAAAGAGAGGTGTCCACAGGTGAGGTTGTAACATT
270
G V H S N I V
INTRON
SIGNAL
J415DIVK1

FIG. 8A-1

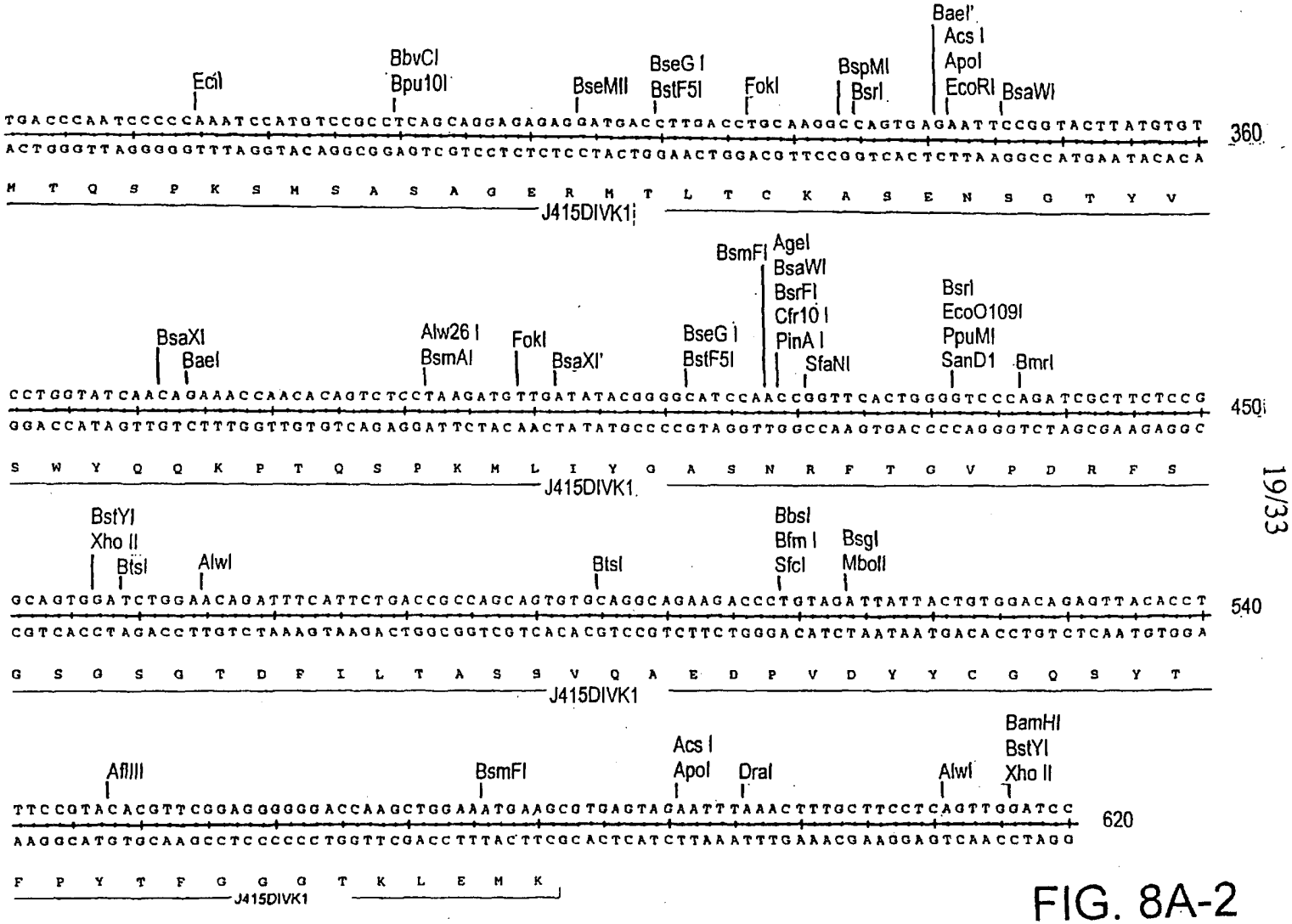

EciI
BbvCI
Bpu10I
BseMII
BseG I
BstF5I
FokI
BspMI
BsrI
BaeI'
Acs I
ApoI
EcoRI
BsaWI
TGACCCAATCCCCCAAATCCATGTCCGCCTCAGCAGGAGAGAGGATGACCTTGACCTGCAAGGCCAGTGAGAATTCCGGTACTTATGTGT
ACTGGGTTAGGGGGTTTAGGTACAGGCGGAGTCGTCCTCTCTCCTACTGGAACTGGACGTTCCGGTCACTCTTAAGGCCATGAATACACA
360
M T Q S P K S M S A S A G E R M T L T C K A S E N S G T Y V
J415DIVK1
BsmFI
AgeI
BsaWI
BsrFI
Cfr10 I
PinA I
SfaNI
BsrI
EcoO109I
PpuMI
SanD1
BmrI
BsaXI
BaeI
Alw26 I
BsmAI
FokI
BsaXI'
BseG I
BstF5I
CCTGGTATCAACAGAAACCAACACAGTCTCCTAAGATGTTGATATACGGGGCATCCAACCGGTTCACTGGGGTCCCAGATCGCTTCTCCG
GGACCATAGTTGTCTTTGGTTGTGTCAGAGGATTCTACAACTATATGCCCCGTAGGTTGGCCAAGTGACCCCAGGGTCTAGCGAAGAGGC
450
S W Y Q Q K P T Q S P K M L I Y G A S N R F T G V P D R F S
J415DIVK1
BstYI
Xho II
BtsI
AlwI
BtsI
BbsI
Bfm I
SfcI
BsgI
MboII
GCAGTGGATCTGGAACAGATTTCATTCTGACCGCCAGCAGTGTGCAGGCAGAAGACCCTGTAGATTATTACTGTGGACAGAGTTACACCT
CGTCACCTAGACCTTGTCTAAAGTAAGACTGGCGGTCGTCACACGTCCGTCTTCTGGGACATCTAATAATGACACCTGTCTCAATGTGGA
540
G S G S G T D F I L T A S S V Q A E D P V D Y Y C G Q S Y T
J415DIVK1
AflIII
BsmFI
Acs I
ApoI
DraI
AlwI
BamHI
BstYI
Xho II
TTCCGTACACGTTCGGAGGGGGGACCAAGCTGGAAATGAAGCGTGAGTAGAATTTAAACTTTGCTTCCTCAGTTGGATCC
AAGGCATGTGCAAGCCTCCCCCCTGGTTCGACCTTTACTTCGCACTCATCTTAAATTTGAAACGAAGGAGTCAACCTAGG
620
F P Y T F G G G T K L E M K
J415DIVK1

FIG. 8A-2

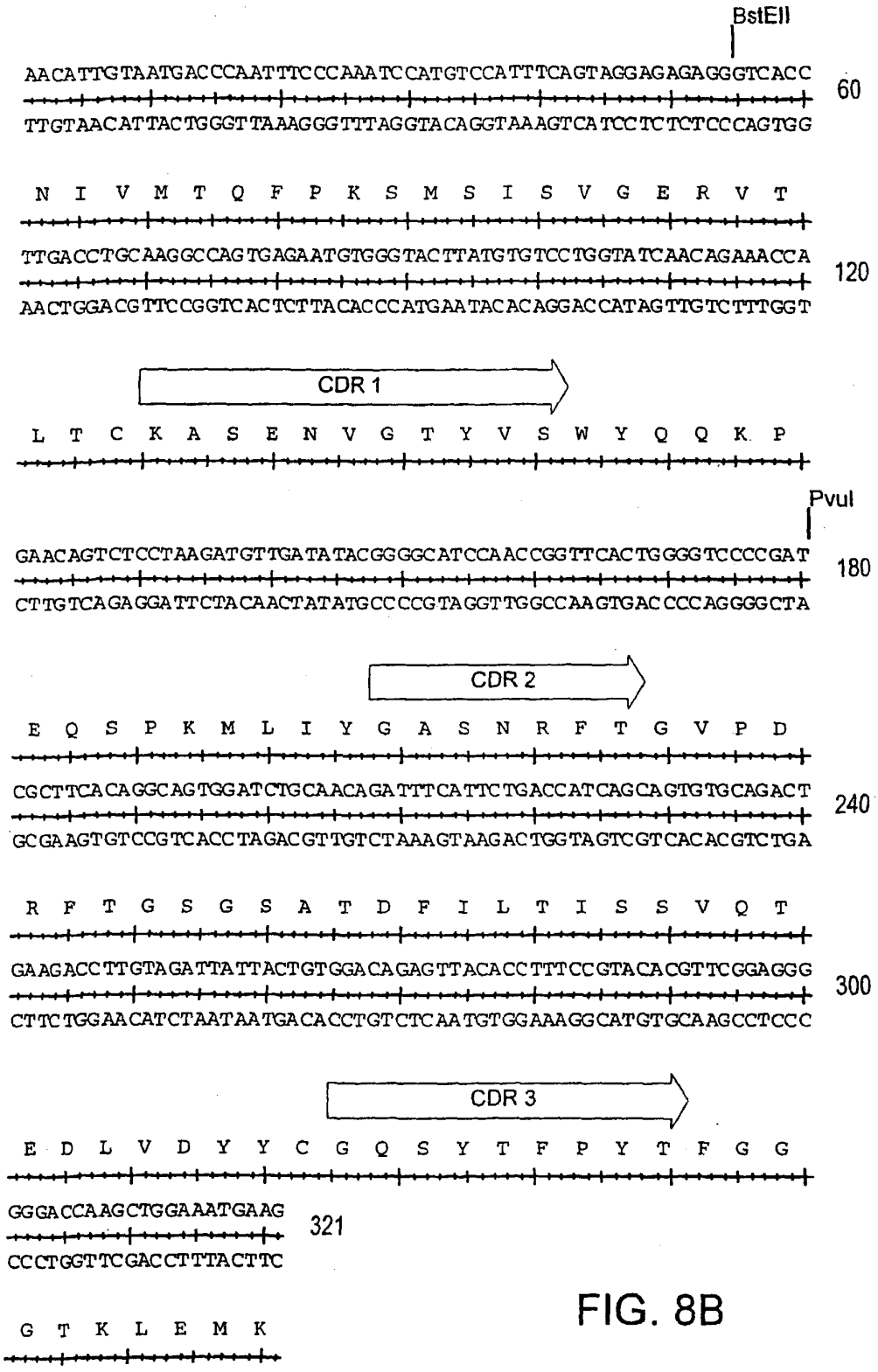

BstEII
AACATTGTAATGACCCAATTTCCCAAATCCATGTCCATTTCAGTAGGAGAGAGGGTCACC
60
TTGTAACATTACTGGGTTAAAGGGTTTAGGTACAGGTAAAGTCATCCTCTCTCCCAGTGG
N I V M T Q F P K S M S I S V G E R V T
TTGACCTGCAAGGCCAGTGAGAATGTGGGTACTTATGTGTCCTGGTATCAACAGAAACCA
120
AACTGGACGTTCCGGTCACTCTTACACCCATGAATACACAGGACCATAGTTGTCTTTGGT
CDR 1
L T C K A S E N V G T Y V S W Y Q Q K P
Pvul
GAACAGTCTCCTAAGATGTTGATATACGGGGCATCCAACCGGTTCACTGGGGTCCCCGAT
180
CTTGTCAGAGGATTCTACAACTATATGCCCCGTAGGTTGGCCAAGTGACCCCAGGGGCTA
CDR 2
E Q S P K M L I Y G A S N R F T G V P D
CGCTTCACAGGCAGTGGATCTGCAACAGATTTCATTCTGACCATCAGCAGTGTGCAGACT
240
GCGAAGTGTCCGTCACCTAGACGTTGTCTAAAGTAAGACTGGTAGTCGTCACACGTCTGA
R F T G S G S A T D F I L T I S S V Q T
GAAGACCTTGTAGATTATTACTGTGGACAGAGTTACACCTTTCCGTACACGTTCGGAGGG
300
CTTCTGGAACATCTAATAATGACACCTGTCTCAATGTGGAAAGGCATGTGCAAGCCTCCC
CDR 3
E D L V D Y Y C G Q S Y T F P Y T F G G
GGGACCAAGCTGGAAATGAAG
321
CCCTGGTTCGACCTTTACTTC
G T K L E M K

FIG. 8B

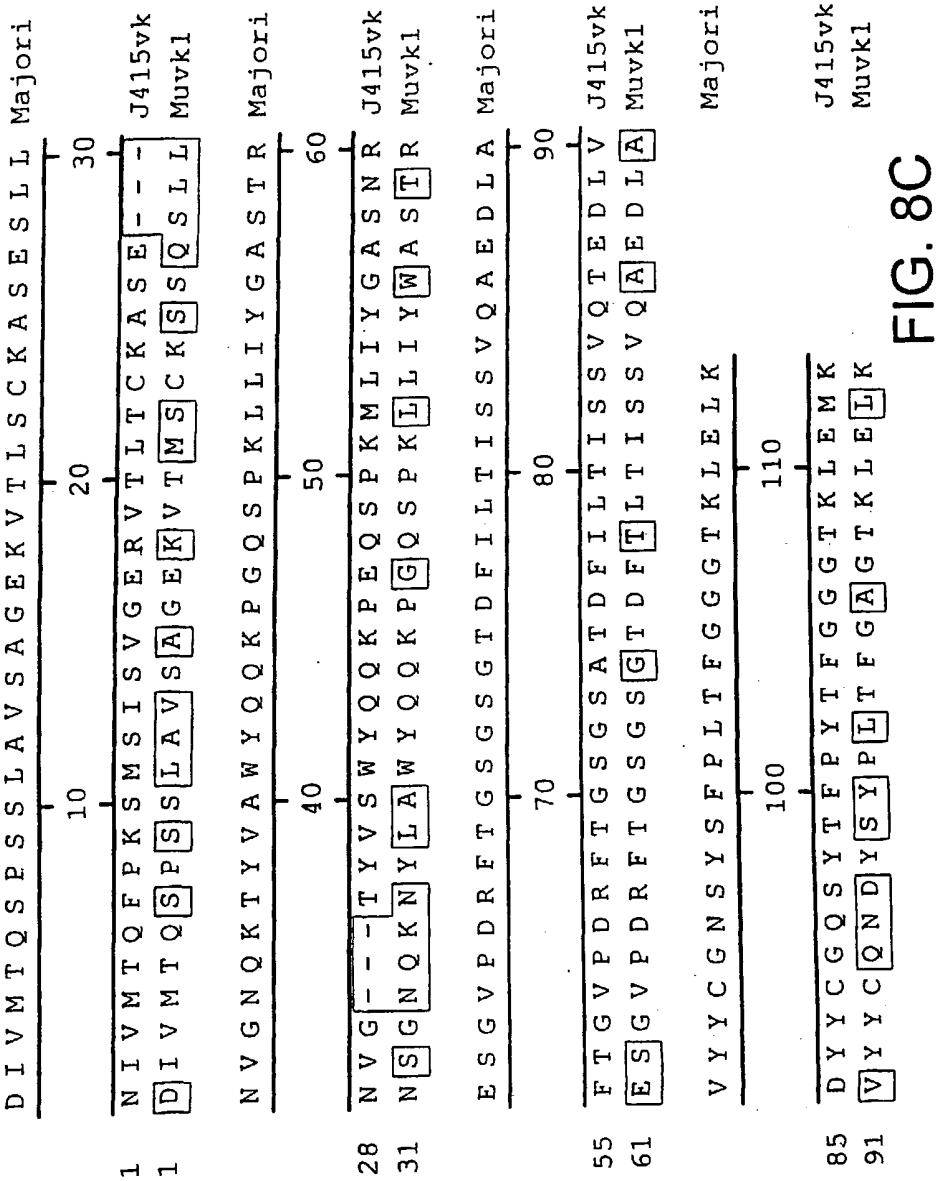

Majori
D I V M T Q S P S S L A V S A G E K V T L S C K A S E S L L
10
20
30
J415vk
1 N I V M T Q F P K S M S I S V G E R V T L T C K A S E - - -
Muvk1
1 D I V M T Q S P S P S L A V S A G E K V T M S C K S Q S L L
Majori
N V G N Q K T Y V A W Y Q Q K P G Q S P K L L I Y G A S T R
40
50
60
J415vk
28 N V G - - - T Y V S W Y Q Q K P E Q S P K M L I Y G A S N R
Muvk1
31 N S G N Q K N Y L A W Y Q Q K P G Q S P K L L I Y W A S T R
Majori
E S G V P D R F T G S G S G T D F I L T I S S V Q A E D L A
70
80
90
J415vk
55 F T G V P D R F T G S G S A T D F I L T I S S V Q T E D L V
Muvk1
61 E S G V P D R F T G S G S G T D F T L T I S S V Q A E D L A
Majori
V Y Y C G N S Y S F P L T F G G G T K L E L K
100
110
J415vk
85 D Y Y C G Q S Y T F P Y T F G G G T K L E M K
Muvk1
91 V Y Y C Q N D Y S Y P L T F G A G T K L E L K

FIG. 8C

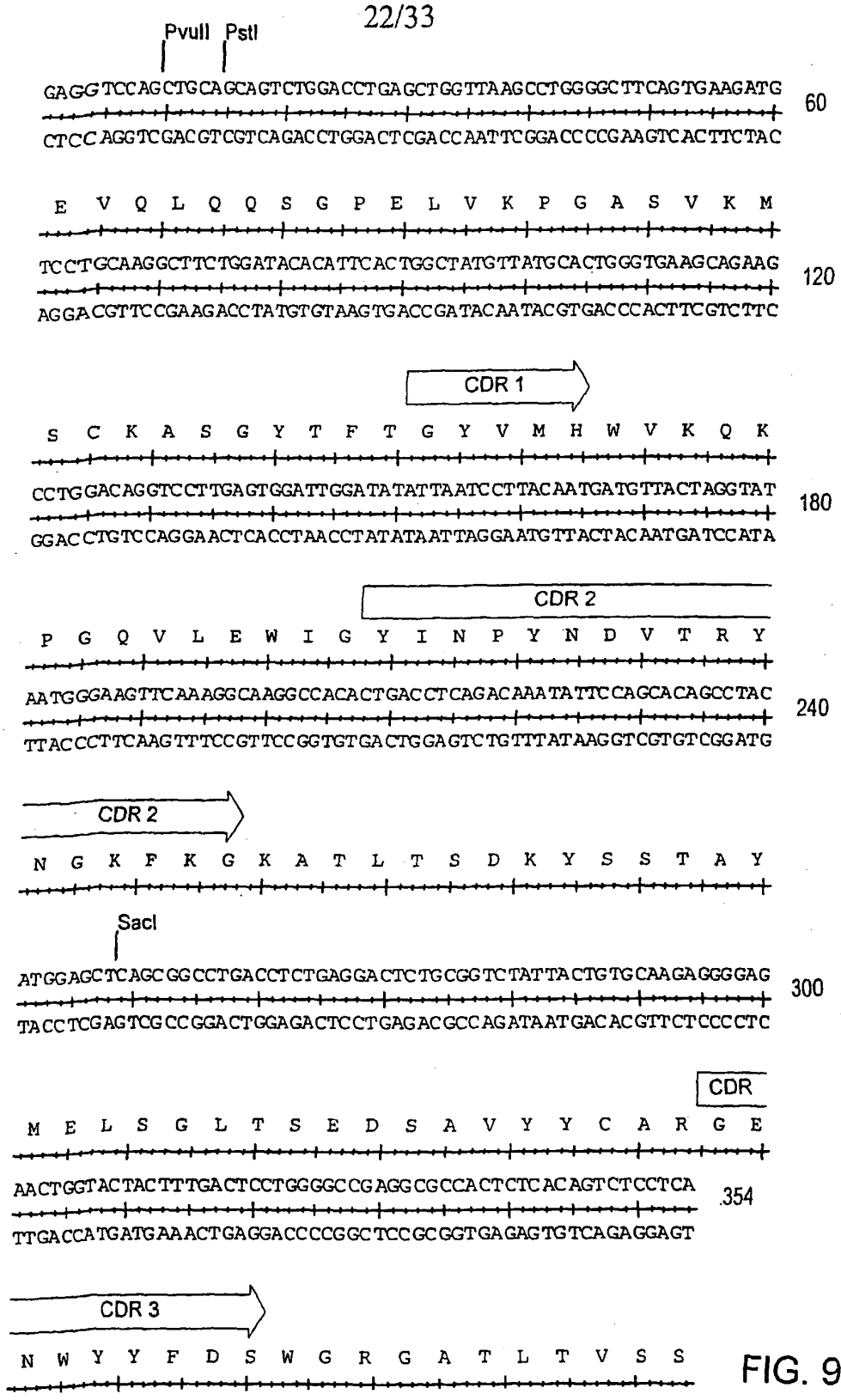

PvuII
PstI
GAGGTCCAGCTGCAGCAGTCTGGACCTGAGCTGGTTAAGCCTGGGGCTTCAGTGAAGATG
60
CTCCAGGTCGACGTCGTCAGACCTGGACTCGACCAATTCGGACCCCGAAGTCACTTCTAC
E V Q L Q Q S G P E L V K P G A S V K M
TCCTGCAAGGCTTCTGGATACACATTCACTGGCTATGTTATGCACTGGGTGAAGCAGAAG
120
AGGACGTTCCGAAGACCTATGTGTAAGTGACCGATACAATACGTGACCCACTTCGTCTTC
CDR 1
S C K A S G Y T F T G Y V M H W V K Q K
CCTGGACAGGTCCTTGAGTGGATTGGATATATTAATCCTTACAATGATGTTACTAGGTAT
180
GGACCTGTCCAGGAACTCACCTAACCTATATAATTAGGAATGTTACTACAATGATCCATA
CDR 2
P G Q V L E W I G Y I N P Y N D V T R Y
AATGGGAAGTTCAAAGGCAAGGCCACACTGACCTCAGACAAATATTCCAGCACAGCCTAC
240
TTACCCTTCAAGTTTCCGTTCCGGTGTGACTGGAGTCTGTTTATAAGGTCGTGTCGGATG
CDR 2
N G K F K G K A T L T S D K Y S S T A Y
SacI
ATGGAGCTCAGCGGCCTGACCTCTGAGGACTCTGCGGTCTATTACTGTGCAAGAGGGGAG
300
TACCTCGAGTCGCCGGACTGGAGACTCCTGAGACGCCAGATAATGACACGTTCTCCCCTC
CDR
M E L S G L T S E D S A V Y Y C A R G E
AACTGGTACTACTTTGACTCCTGGGGCCGAGGCGCCACTCTCACAGTCTCCTCA
354
TTGACCATGATGAAACTGAGGACCCCGGCTCCGCGGTGAGAGTGTCAGAGGAGT
CDR 3
N W Y Y F D S W G R G A T L T V S S

FIG. 9A

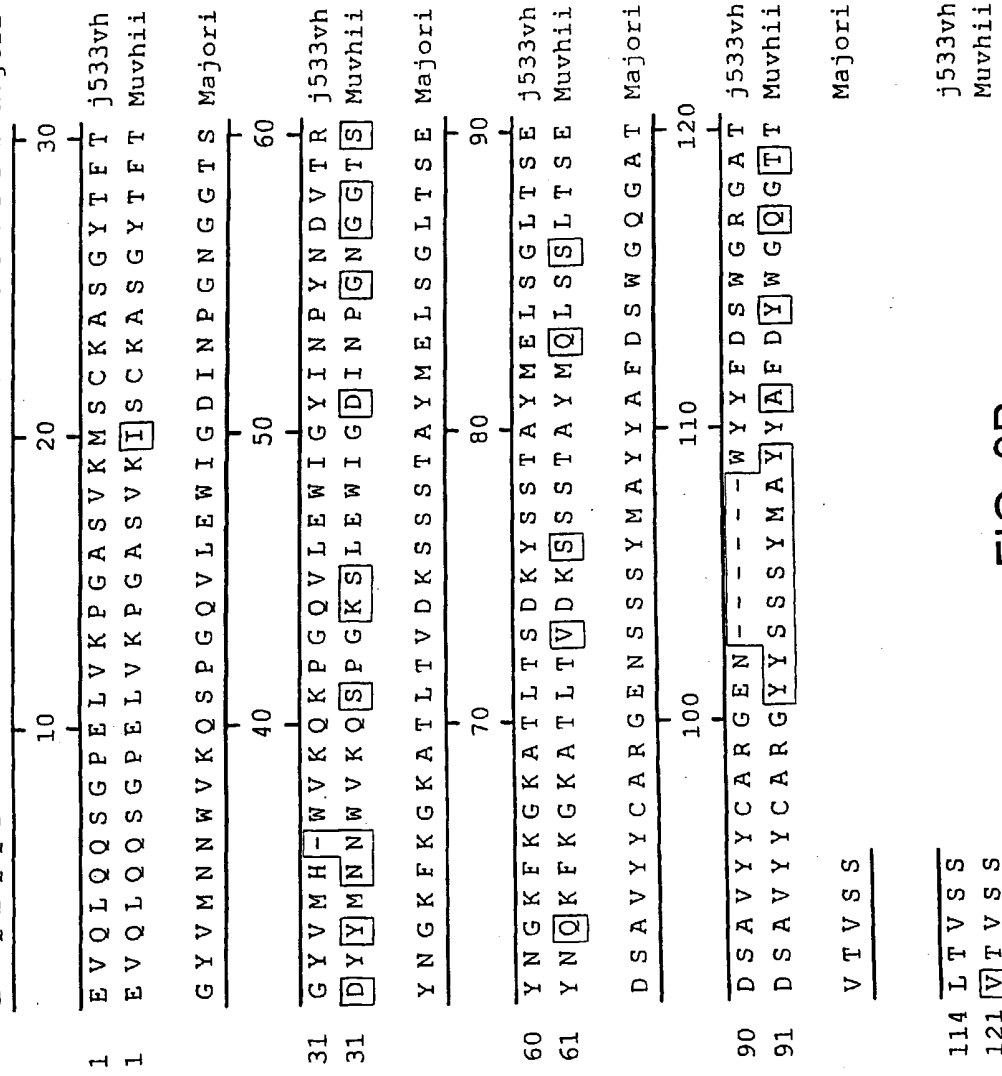


FIG. 9B

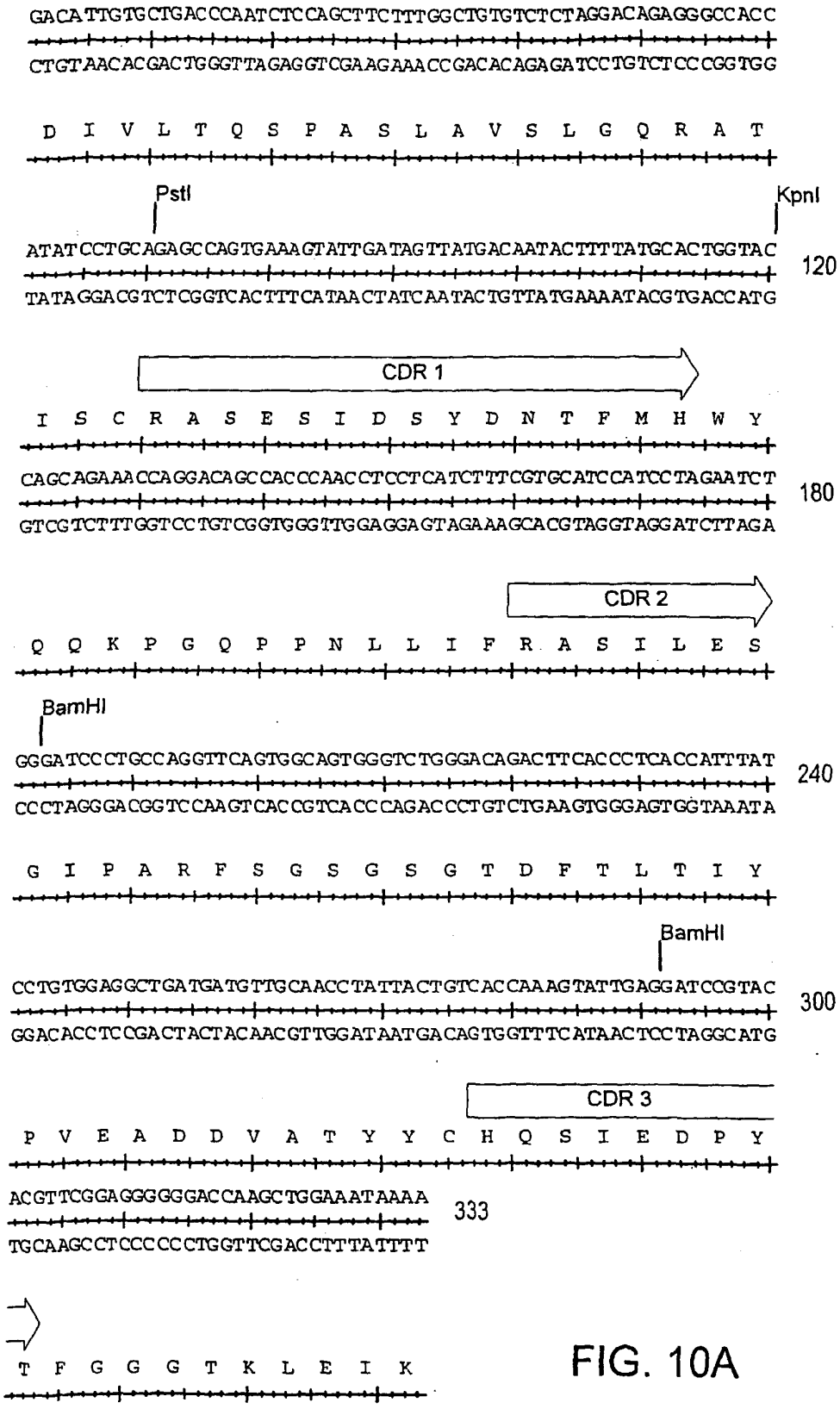

GACATTGTGCTGACCCAATCTCCAGCTTCTTTGGCTGTGTCTCTAGGACAGAGGGCCACC
CTGTAACACGACTGGGTTAGAGGTCGAAGAAACCGACACAGAGATCCTGTCTCCCGGTGG
D I V L T Q S P A S L A V S L G Q R A T
PstI
KpnI
ATATCCTGCAGAGCCAGTGAAAGTATTGATAGTTATGACAATACTTTTATGCACTGGTAC
120
TATAGGACGTCTCGGTCACTTTCATAACTATCAATACTGTTATGAAAATACGTGACCATG
CDR 1
I S C R A S E S I D S Y D N T F M H W Y
CAGCAGAAACCAGGACAGCCACCCAACCTCCTCATCTTTCGTGCATCCATCCTAGAATCT
180
GTCGTCTTTGGTCCTGTCGGTGGGTTGGAGGAGTAGAAAGCACGTAGGTAGGATCTTAGA
CDR 2
Q Q K P G Q P P N L L I F R A S I L E S
BamHI
GGGATCCCTGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACCCTCACCATTTAT
240
CCCTAGGGACGGTCCAAGTCACCGTCACCCAGACCCTGTCTGAAGTGGGAGTGGTAAATA
G I P A R F S G S G S G T D F T L T I Y
BamHI
CCTGTGGAGGCTGATGATGTTGCAACCTATTACTGTCACCAAAGTATTGAGGATCCGTAC
300
GGACACCTCCGACTACTACAACGTTGGATAATGACAGTGGTTTCATAACTCCTAGGCATG
CDR 3
P V E A D D V A T Y Y C H Q S I E D P Y
ACGTTCGGAGGGGGGACCAAGCTGGAAATAAAA
333
TGCAAGCCTCCCCCCTGGTTCGACCTTTATTTT
T F G G G T K L E I K

FIG. 10A

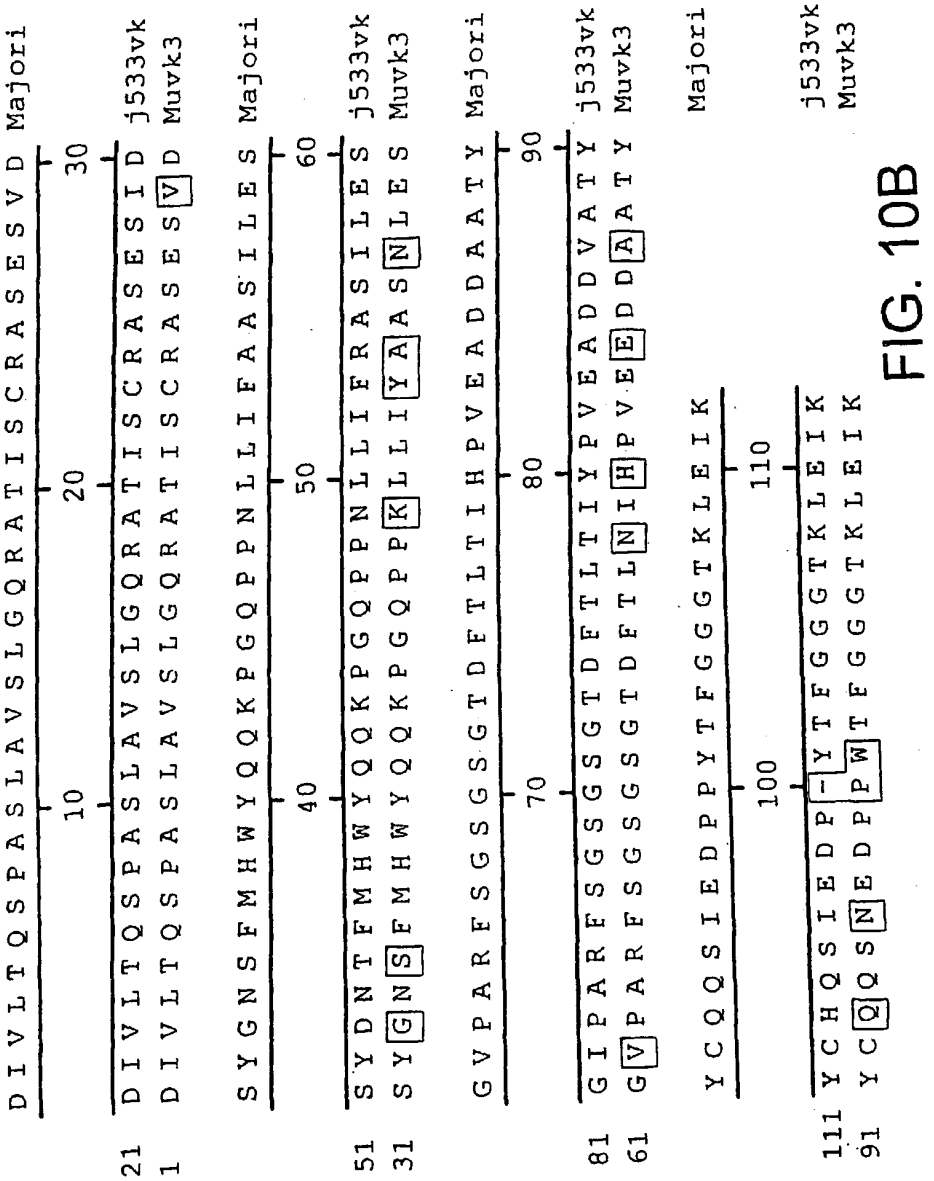

D I V L T Q S P A S L A V S L G Q R A T I S C R A S E S V D Majori
10 20 30
21 D I V L T Q S P A S L A V S L G Q R A T I S C R A S E S I D j533vk
1 D I V L T Q S P A S L A V S L G Q R A T I S C R A S E S V D Muvk3
S Y G N S F M H W Y Q Q K P G Q P P N L L I F A A S I L E S Majori
40 50 60
51 S Y D N T F M H W Y Q Q K P G Q P P N L L I F R A S I L E S j533vk
31 S Y G N S F M H W Y Q Q K P G Q P P K L L I Y A A S N L E S Muvk3
G V P A R F S G S G S G T D F T L T I H P V E A D D A A T Y Majori
70 80 90
81 G I P A R F S G S G S G T D F T L T I Y P V E A D D V A T Y j533vk
61 G V P A R F S G S G S G T D F T L N I H P V E E D D A A T Y Muvk3
Y C Q Q S I E D P P Y T F G G G T K L E I K Majori
100 110
111 Y C H Q S I E D P - Y T F G G G T K L E I K j533vk
91 Y C Q Q S N E D P P W T F G G G T K L E I K Muvk3

FIG. 10B

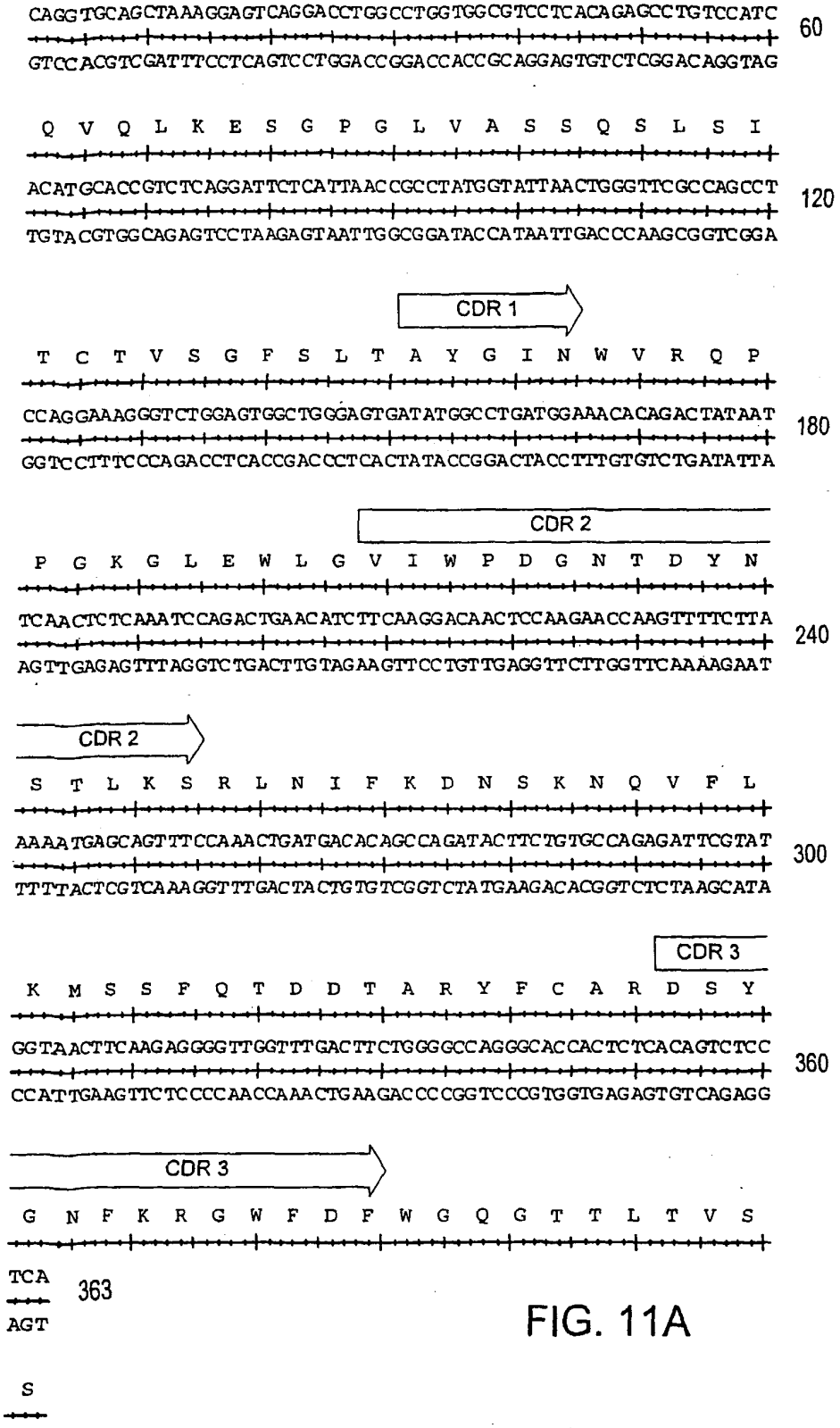

CAGGTGCAGCTAAAGGAGTCAGGACCTGGCCTGGTGGCGTCCTCACAGAGCCTGTCCATC
60
GTCCACGTCGATTTCCTCAGTCCTGGACCGGACCACCGCAGGAGTGTCTCGGACAGGTAG
Q V Q L K E S G P G L V A S S Q S L S I
ACATGCACCGTCTCAGGATTCTCATTAACCGCCTATGGTATTAACTGGGTTCGCCAGCCT
120
TGTACGTGGCAGAGTCCTAAGAGTAATTGGCGGATACCATAATTGACCCAAGCGGTCGGA
CDR 1
T C T V S G F S L T A Y G I N W V R Q P
CCAGGAAAGGGTCTGGAGTGGCTGGGAGTGATATGGCCTGATGGAAACACAGACTATAAT
180
GGTCCTTTCCCAGACCTCACCGACCCTCACTATACCGGACTACCTTTGTGTCTGATATTA
CDR 2
P G K G L E W L G V I W P D G N T D Y N
TCAACTCTCAAATCCAGACTGAACATCTTCAAGGACAACTCCAAGAACCAAGTTTTCTTA
240
AGTTGAGAGTTTAGGTCTGACTTGTAGAAGTTCCTGTTGAGGTTCTTGGTTCAAAAGAAT
CDR 2
S T L K S R L N I F K D N S K N Q V F L
AAAATGAGCAGTTTCCAAACTGATGACACAGCCAGATACTTCTGTGCCAGAGATTCGTAT
300
TTTTACTCGTCAAAGGTTTGACTACTGTGTCGGTCTATGAAGACACGGTCTCTAAGCATA
CDR 3
K M S S F Q T D D T A R Y F C A R D S Y
GGTAACTTCAAGAGGGGTTGGTTTGACTTCTGGGGCCAGGGCACCACTCTCACAGTCTCC
360
CCATTGAAGTTCTCCCCAACCAAACTGAAGACCCCGGTCCCGTGGTGAGAGTGTCAGAGG
CDR 3
G N F K R G W F D F W G Q G T T L T V S
TCA
AGT
363
S

FIG. 11A

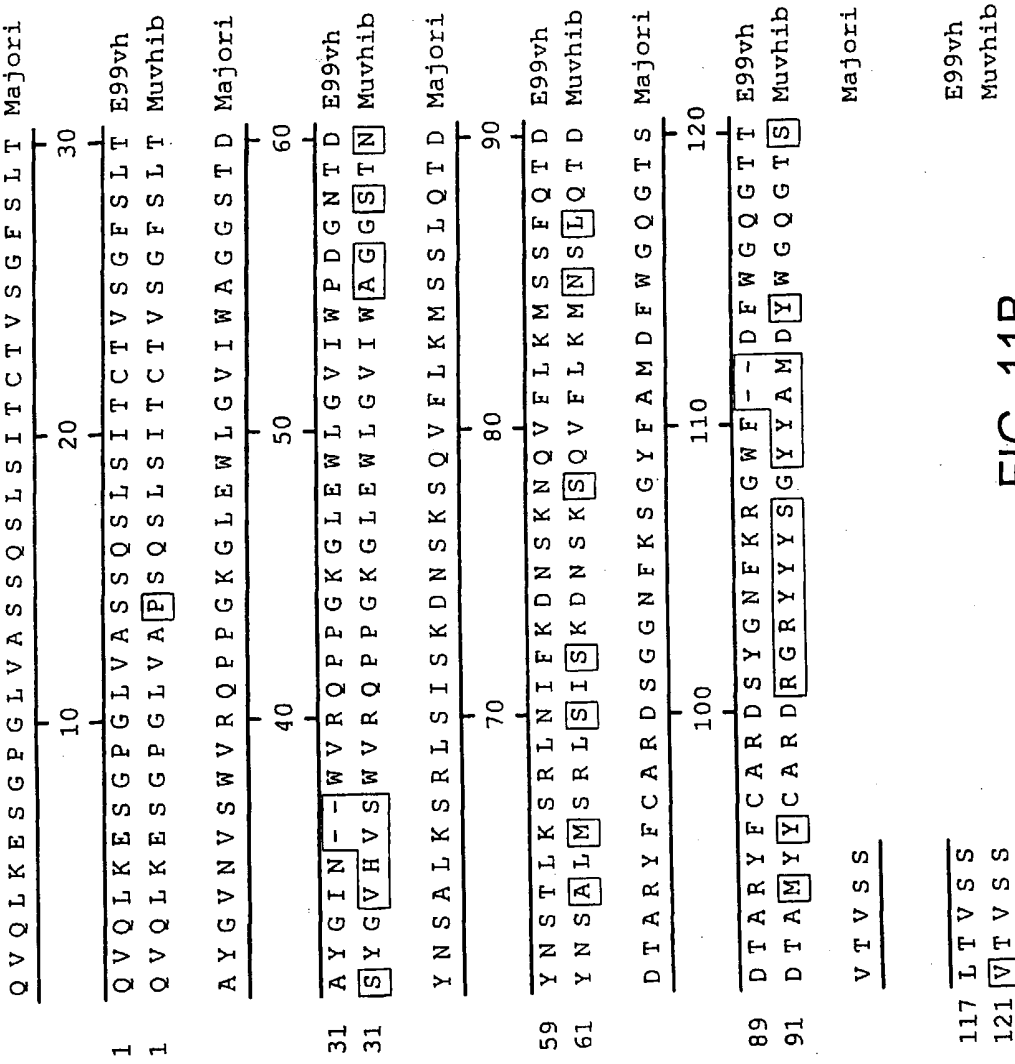

Majori
E99vh
Muvhib
10
20
30
40
50
60
70
80
90
100
110
120
Q V Q L K E S G P G L V A S S Q S L S I T C T V S G F S L T
Q V Q L K E S G P G L V A P S Q S L S I T C T V S G F S L T
A Y G V N W V R Q P P G K G L E W L G V I W A G G S T D
A Y G I N W V R Q P P G K G L E W L G V I W P D G N T D
S Y G V H V S W V R Q P P G K G L E W L G V I W A G G S T N
Y N S A L K S R L S I S K D N S K S Q V F L K M S S L Q T D
Y N S T L K S R L N I F K D N S K N Q V F L K M S S F Q T D
Y N S A L M S R L S I S K D N S K S Q V F L K M N S L Q T D
D T A R Y F C A R D S G G N F K S G Y F A M D F W G Q G T S
D T A R Y F C A R D S Y G N F K R G W F - - D F W G Q G T T
D T A M Y Y C A R D R G R Y Y Y S G Y Y A M D Y W G Q G T S
V T V S S
117 L T V S S
121 V T V S S

FIG. 11B

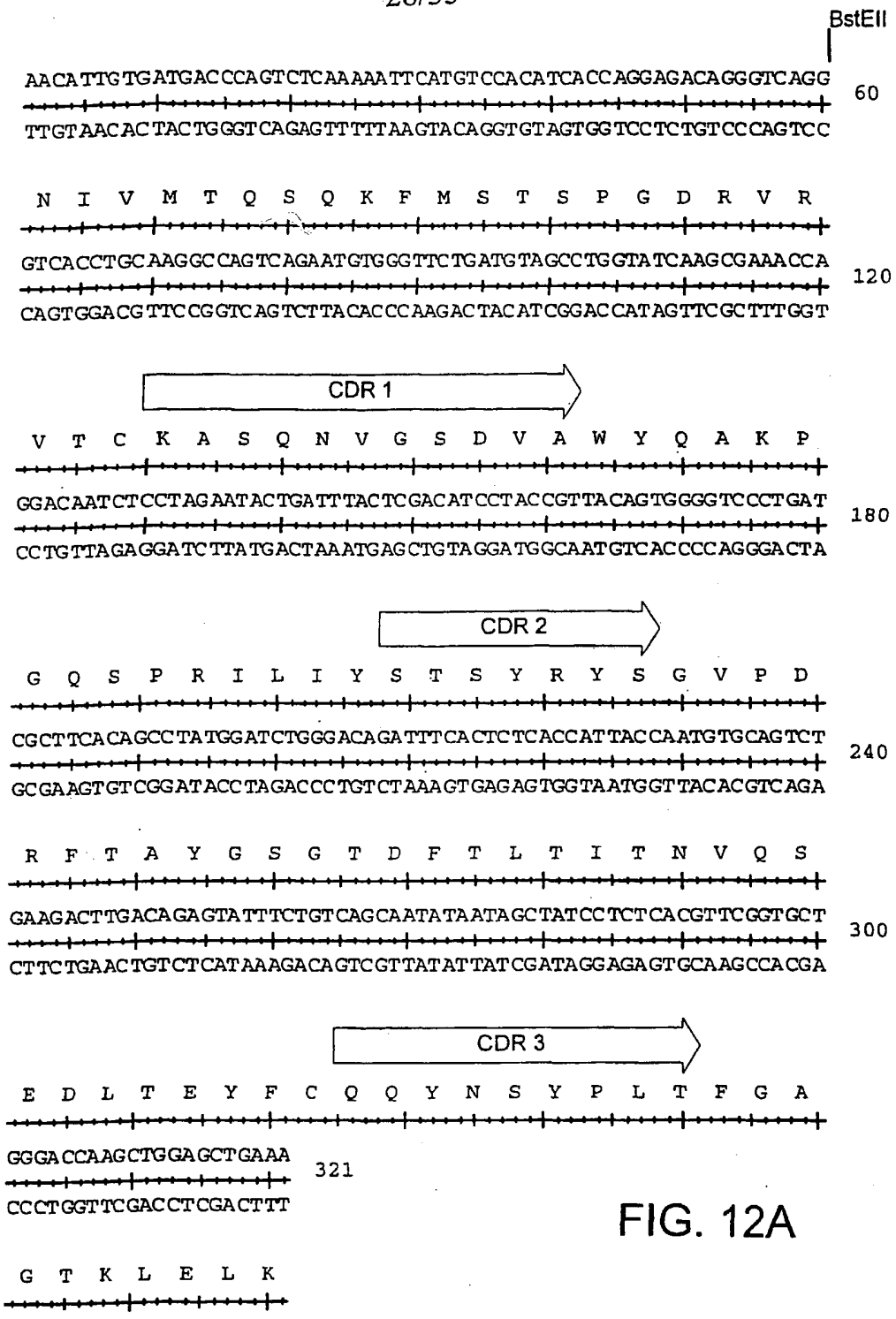

BstEII
AACATTGTGATGACCCAGTCTCAAAAATTCATGTCCACATCACCAGGAGACAGGGTCAGG
TTGTAACACTACTGGGTCAGAGTTTTTAAGTACAGGTGTAGTGGTCCTCTGTCCCAGTCC
60
N I V M T Q S Q K F M S T S P G D R V R
GTCACCTGCAAGGCCAGTCAGAATGTGGGTTCTGATGTAGCCTGGTATCAAGCGAAACCA
CAGTGGACGTTCCGGTCAGTCTTACACCCAAGACTACATCGGACCATAGTTCGCTTTGGT
120
CDR 1
V T C K A S Q N V G S D V A W Y Q A K P
GGACAATCTCCTAGAATACTGATTTACTCGACATCCTACCGTTACAGTGGGGTCCCTGAT
CCTGTTAGAGGATCTTATGACTAAATGAGCTGTAGGATGGCAATGTCACCCCAGGGACTA
180
CDR 2
G Q S P R I L I Y S T S Y R Y S G V P D
CGCTTCACAGCCTATGGATCTGGGACAGATTTCACTCTCACCATTACCAATGTGCAGTCT
GCGAAGTGTCGGATACCTAGACCCTGTCTAAAGTGAGAGTGGTAATGGTTACACGTCAGA
240
R F T A Y G S G T D F T L T I T N V Q S
GAAGACTTGACAGAGTATTTCTGTCAGCAATATAATAGCTATCCTCTCACGTTCGGTGCT
CTTCTGAACTGTCTCATAAAGACAGTCGTTATATTATCGATAGGAGAGTGCAAGCCACGA
300
CDR 3
E D L T E Y F C Q Q Y N S Y P L T F G A
GGGACCAAGCTGGAGCTGAAA
CCCTGGTTCGACCTCGACTTT
321
G T K L E L K

FIG. 12A

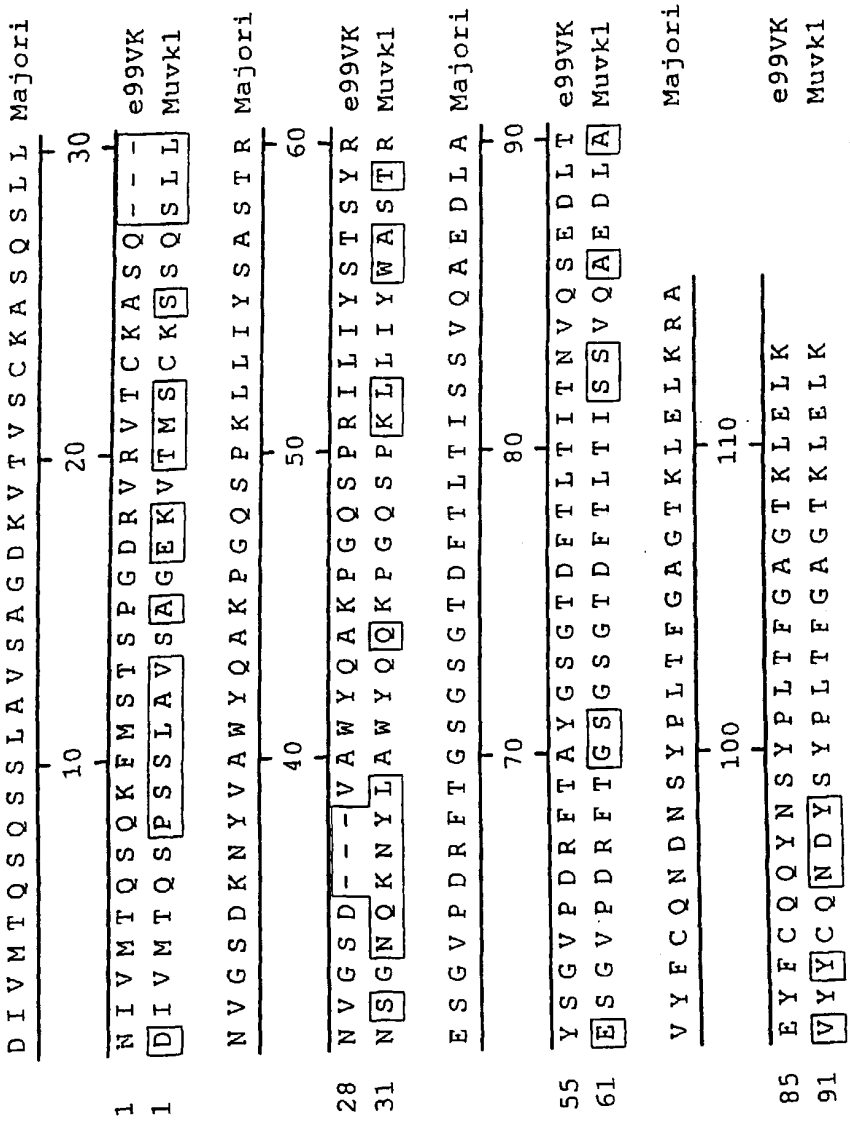

Majori
10
20
30
e99VK
Muvk1
Majori
40
50
60
e99VK
Muvk1
Majori
70
80
90
e99VK
Muvk1
Majori
100
110
e99VK
Muvk1

FIG. 12B

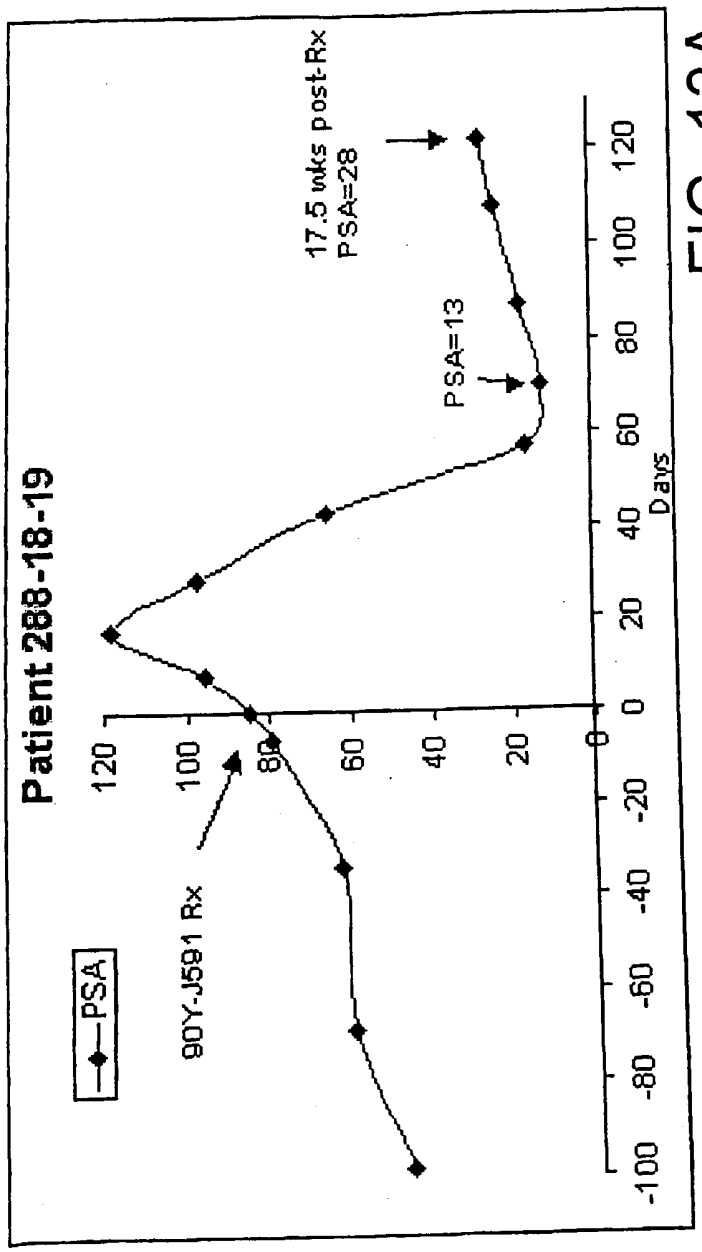

Patient 288-18-19
PSA
90Y-J591 Rx
PSA=13
17.5 wks post-Rx
PSA=28
Days
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
20
40
60
80
100
120

FIG. 13A

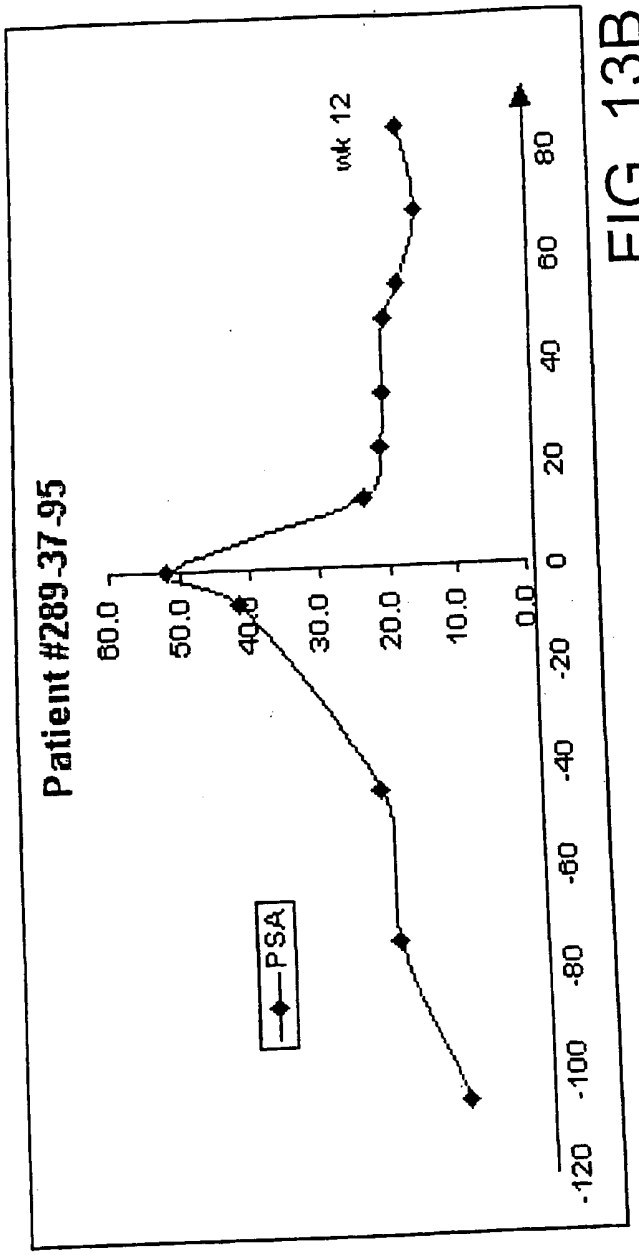

Patient #289-37-95
PSA
wk 12
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-120
-100
-80
-60
-40
-20
0
20
40
60
80

FIG. 13B

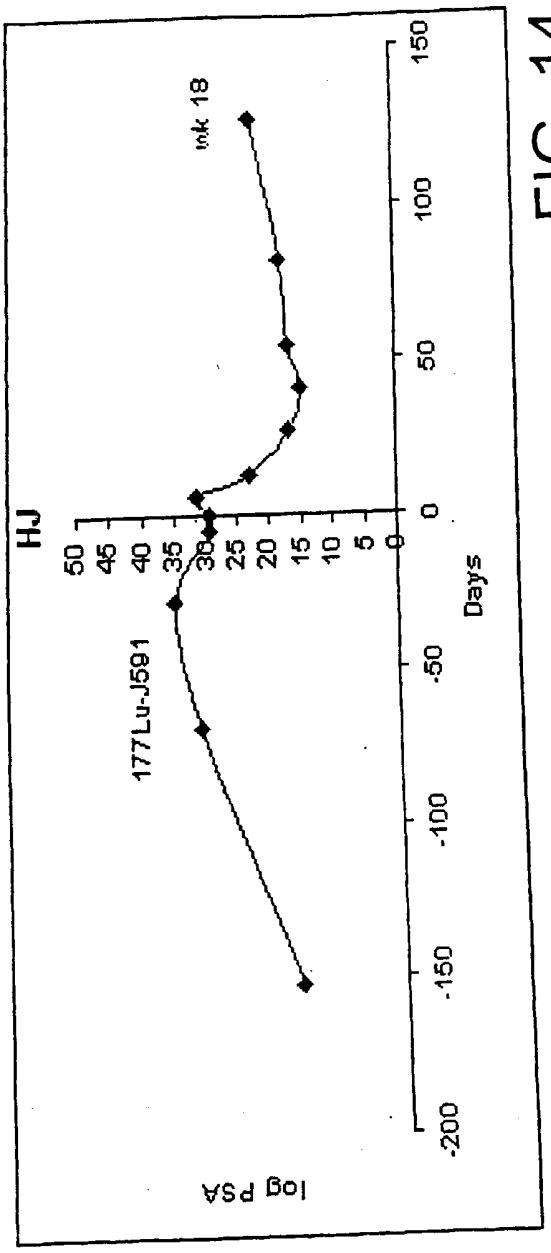
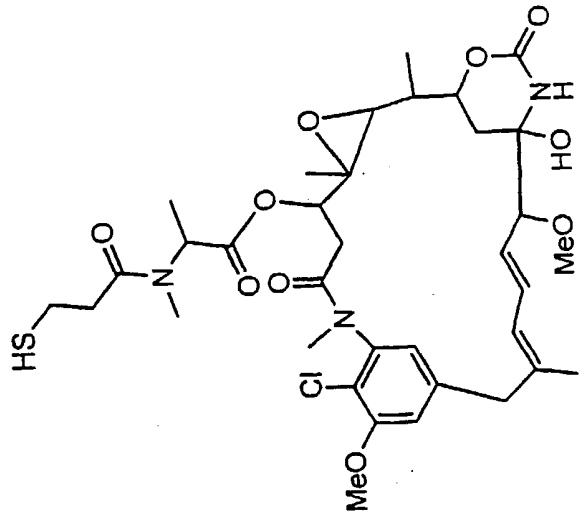
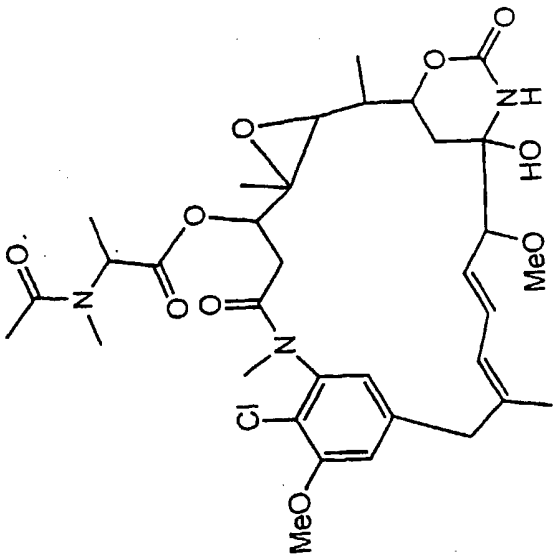

HJ
177Lu-J591
mk 18
log PSA
Days
50
45
40
35
30
25
20
15
10
5
0
150
100
50
0
-50
-100
-150
-200

FIG. 14



$C_{35}H_{48}ClN_3O_{10}S$
Exact Mass: 737.27
Mol. Wt.: 738.29
DM1



$C_{34}H_{46}ClN_3O_{10}$
Exact Mass: 691.29
Mol. Wt.: 692.20
Maytansine

FIG. 15

SEQUENCE LISTING

<110> Cornell Research Foundation, Inc.

<120> MODIFIED ANTIBODIES TO PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND USES THEREOF

<130> 10448-163WO1

<150> 60/323,585
<151> 2001-09-20

<150> 60/362,810
<151> 2002-03-08

<150> 60/295,214
<151> 2001-06-01

<160> 128

<170> FastSEQ for Windows Version 4.0

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<213> Mus musculus

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1 5 10

<210> 2
<211> 17
<212> PRT
<213> Mus musculus

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1 5 10 15
Asp

<210> 3
<211> 6
<212> PRT
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1 5

<210> 4
<211> 11
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<210> 5
<211> 7
<212> PRT
<213> Mus musculus

<400> 5
Trp Ala Ser Thr Arg His Thr
1 5

<210> 6
<211> 9
<212> PRT
<213> Mus musculus

<400> 6
Gln Gln Tyr Asn Ser Tyr Pro Leu Thr
1 5

<210> 7
<211> 82
<212> PRT
<213> Mus musculus

<400> 7
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Ser Val Arg Ile Ser Cys Lys Thr Ser Trp Val Lys Gln Ser His Gly
20 25 30
Lys Ser Leu Glu Trp Ile Gly Lys Ala Thr Leu Thr Val Asp Lys Ser
35 40 45
Ser Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser
50 55 60
Ala Val Tyr Tyr Cys Ala Ala Trp Gly Gln Gly Thr Thr Leu Thr Val
65 70 75 80
Ser Ser

<210> 8
<211> 80
<212> PRT
<213> Mus musculus

<400> 8
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1 5 10 15
Asp Arg Val Ser Ile Ile Cys Trp Tyr Gln Gln Lys Pro Gly Gln Ser
20 25 30
Pro Lys Leu Leu Ile Tyr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly
35 40 45
Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser Glu Asp
50 55 60
Leu Ala Asp Tyr Phe Cys Phe Gly Ala Gly Thr Met Leu Asp Leu Lys
65 70 75 80

<210> 9
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J591

<400> 9
Glu Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Thr Ser
20 25

<210> 10
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J591

<400> 10
Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly
1 5 10

<210> 11
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J591

<400> 11
Lys Ala Thr Leu Thr Val Asp Lys Ser Thr Asp Thr Ala Tyr Met Glu
1 5 10 15
Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 12
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J591

<400> 12
Trp Gly Gln Gly Thr Leu Leu Thr Val Ser Ser
1 5 10

<210> 13
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J591

<400> 13
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Thr Ser Val Gly
1 5 10 15
Asp Arg Val Thr Leu Thr Cys
20

<210> 14
<211> 15

<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J591

<400> 14
Trp Tyr Gln Gln Lys Pro Gly Pro Ser Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 15
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J591

<400> 15
Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15
Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Asp Tyr Tyr Cys
20 25 30

<210> 16
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J591

<400> 16
Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
1 5 10

<210> 17
<211> 82
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J591

<400> 17
Glu Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Thr Ser Trp Val Lys Gln Ala Pro Gly
20 25 30
Lys Gly Leu Glu Trp Ile Gly Lys Ala Thr Leu Thr Val Asp Lys Ser
35 40 45
Thr Asp Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr
50 55 60
Ala Val Tyr Tyr Cys Ala Ala Trp Gly Gln Gly Thr Leu Leu Thr Val
65 70 75 80
Ser Ser

<210> 18
<211> 80
<212> PRT

<213> Artificial Sequence

<220>
<223> deimmunized light chain J591

<400> 18
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Thr Ser Val Gly
1 5 10 15
Asp Arg Val Thr Leu Thr Cys Trp Tyr Gln Gln Lys Pro Gly Pro Ser
20 25 30
Pro Lys Leu Leu Ile Tyr Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly
35 40 45
Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
50 55 60
Phe Ala Asp Tyr Tyr Cys Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
65 70 75 80

<210> 19
<211> 115
<212> PRT
<213> Mus musculus

<400> 19
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Thr
1 5 10 15
Ser Val Arg Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr
20 25 30
Thr Ile His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45
Gly Asn Ile Asn Pro Asn Asn Gly Gly Thr Thr Tyr Asn Gln Lys Phe
50 55 60
Glu Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Ala Gly Trp Asn Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr
100 105 110
Val Ser Ser
115

<210> 20
<211> 107
<212> PRT
<213> Mus musculus

<400> 20
Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15
Asp Arg Val Ser Ile Ile Cys Lys Ala Ser Gln Asp Val Gly Thr Ala
20 25 30
Val Asp Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45
Tyr Trp Ala Ser Thr Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
65 70 75 80
Glu Asp Leu Ala Asp Tyr Phe Cys Gln Gln Tyr Asn Ser Tyr Pro Leu
85 90 95
Thr Phe Gly Ala Gly Thr Met Leu Asp Leu Lys
100 105

<210> 21
<211> 115
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J591

<400> 21
Glu Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr
20 25 30
Thr Ile His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Asn Ile Asn Pro Asn Asn Gly Gly Thr Thr Tyr Asn Gln Lys Phe
50 55 60
Glu Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Thr Asp Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Ala Gly Trp Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Leu Thr
100 105 110
Val Ser Ser
115

<210> 22
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J591

<400> 22
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Thr Ser Val Gly
1 5 10 15
Asp Arg Val Thr Leu Thr Cys Lys Ala Ser Gln Asp Val Gly Thr Ala
20 25 30
Val Asp Trp Tyr Gln Gln Lys Pro Gly Pro Ser Pro Lys Leu Leu Ile
35 40 45
Tyr Trp Ala Ser Thr Arg His Thr Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Asp Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro Leu
85 90 95
Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
100 105

<210> 23
<211> 807
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (122)...(166)

<221> CDS
<222> (249)...(605)

<223> deimmunized heavy chain J591

<400> 23
aagcttatga atatgcaaat cctctgaatc tacatggtaa atataggttt gtctatacca 60
caaacagaaa aacatgagat cacagttctc tctacagtta ctgagcacac aggacctcac 120
c atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca 166
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
1 5 10 15

ggtaaggggc tcacagtagc aggcttgagg tctggacata tatatgggtg acaatgacat 226
ccactttgcc tttctctcca ca ggt gtc cac tcc gag gtc caa ctg gta cag 278
Gly Val His Ser Glu Val Gln Leu Val Gln
20 25

tct gga cct gaa gtg aag aag cct ggg gct aca gtg aag ata tcc tgc 326
Ser Gly Pro Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser Cys
30 35 40

aag act tct gga tac aca ttc act gaa tat acc ata cac tgg gtg aag 374
Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr Thr Ile His Trp Val Lys
45 50 55

cag gcc cct gga aag ggc ctt gag tgg att gga aac atc aat cct aac 422
Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Asn Ile Asn Pro Asn
60 65 70

aat ggt ggt acc acc tac aat cag aag ttc gag gac aag gcc aca cta 470
Asn Gly Gly Thr Thr Tyr Asn Gln Lys Phe Glu Asp Lys Ala Thr Leu
75 80 85

act gta gac aag tcc acc gat aca gcc tac atg gag ctc agc agc cta 518
Thr Val Asp Lys Ser Thr Asp Thr Ala Tyr Met Glu Leu Ser Ser Leu
90 95 100 105

aga tct gag gat act gca gtc tat tat tgt gca gct ggt tgg aac ttt 566
Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Gly Trp Asn Phe
110 115 120

gac tac tgg ggc caa ggg acc ctg ctc acc gtc tcc tca ggtgagtcct 615
Asp Tyr Trp Gly Gln Gly Thr Leu Leu Thr Val Ser Ser
125 130

tacaacctct ctcttctatt cagcttaaat agattttact gcatttgttg gggggaaat 675
gtgtgtatct gaatttcagg tcatgaagga ctagggacac cttgggagtc agaaagggtc 735
attgggagcc cgggctgatg cagacagaca tcctcagctc ccagacttca tggccagaga 795
tttataggat cc 807

<210> 24
<211> 807
<212> DNA
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J591

<400> 24
ggatcctata aatctctggc catgaagtct gggagctgag gatgtctgtc tgcatcagcc 60
cgggctccca atgacccttt ctgactccca aggtgtccct agtccttcat gacctgaaat 120
tcagatacac acatttcccc cccaacaaat gcagtaaaat ctatttaagc tgaatagaag 180
agagaggttg taaggactca cctgaggaga cggtgagcag ggtcccttgg ccccagtagt 240

caaagttcca accagctgca caataataga ctgcagtatc ctcagatctt aggctgctga 300
gctccatgta ggctgtatcg gtggacttgt ctacagttag tgtggccttg tcctcgaact 360
tctgattgta ggtggtacca ccattgttag gattgatgtt tccaatccac tcaaggccct 420
ttccaggggc ctgcttcacc cagtgtatgg tatattcagt gaatgtgtat ccagaagtct 480
tgcaggatat cttcactgta gccccaggct tcttcacttc aggtccagac tgtaccagtt 540
ggacctcgga gtggacacct gtggagagaa aggcaaagtg gatgtcattg tcacccatat 600
atatgtccag acctcaagcc tgctactgtg agccccttac ctgtagctgt tgctaccaag 660
aagaggatga tacagctcca tcccatggtg aggtcctgtg tgctcagtaa ctgtagagag 720
aactgtgatc tcatgttttt ctgtttgtgg tatagacaaa cctatattta ccatgtagat 780
tcagaggatt tgcatattca taagctt 807

<210> 25
<211> 620
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (122)...(166)

<221> CDS
<222> (249)...(581)

<223> deimmunized light chain J591

<400> 25
aagcttatga atatgcaaat cctctgaatc tacatggtaa atataggttt gtctatacca 60
caaacagaaa aacatgagat cacagttctc tctacagtta ctgagcacac aggacctcac 120
c atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca 166
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
1 5 10 15

ggtaaggggc tcacagtagc aggcttgagg tctggacata tatatgggtg acaatgacat 226
ccactttgcc tttctctcca ca ggt gtc cac tcc gac atc cag atg acc cag 278
Gly Val His Ser Asp Ile Gln Met Thr Gln
20 25

tct ccc tca tcc ctg tcc aca tca gta gga gac agg gtc acc ctc acc 326
Ser Pro Ser Ser Leu Ser Thr Ser Val Gly Asp Arg Val Thr Leu Thr
30 35 40

tgt aag gcc agt caa gat gtg ggt act gct gta gac tgg tat caa cag 374
Cys Lys Ala Ser Gln Asp Val Gly Thr Ala Val Asp Trp Tyr Gln Gln
45 50 55

aaa cca gga cca tct cct aaa cta ctg att tat tgg gca tcc act cgg 422
Lys Pro Gly Pro Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
60 65 70

cac act gga atc cct agt cgc ttc tca ggc agt gga tct ggg aca gac 470
His Thr Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
75 80 85

ttc act ctc acc att tct agt ctt cag cct gaa gac ttt gca gat tat 518
Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Asp Tyr
90 95 100 105

tac tgt cag caa tat aac agc tat cct ctc acg ttc ggt cct ggg acc 566
Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro Leu Thr Phe Gly Pro Gly Thr
110 115 120

aag gtg gac atc aaa cgtgagtaga atttaaactt tgcttcctca gttggatcc 620
Lys Val Asp Ile Lys
125

<210> 26
<211> 620
<212> DNA
<213> Artificial Sequence

<220>
<223> deimmunized light chain J591

<400> 26
ggatccaact gaggaagcaa agtttaaatt ctactcacgt ttgatgtcca ccttggtccc 60
aggaccgaac gtgagaggat agctgttata ttgctgacag taataatctg caaagtcttc 120
aggctgaaga ctagaaatgg tgagagtgaa gtctgtccca gatccactgc ctgagaagcg 180
actagggatt ccagtgtgcc gagtggatgc ccaataaatc agtagtttag gagatggtcc 240
tggtttctgt tgataccagt ctacagcagt acccacatct tgactggcct tacaggtgag 300
ggtgaccctg tctcctactg atgtggacag ggatgaggga gactgggtca tctggatgtc 360
ggagtggaca cctgtggaga gaaaggcaaa gtggatgtca ttgtcaccca tatatatgtc 420
cagacctcaa gcctgctact gtgagcccct tacctgtagc tgttgctacc aagaagagga 480
tgatacagct ccatcccatg gtgaggtcct gtgtgctcag taactgtaga gagaactgtg 540
atctcatgtt tttctgtttg tggtatagac aaacctatat ttaccatgta gattcagagg 600
atttgcatat tcataagctt 620

<210> 27
<211> 134
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J591

<400> 27
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15
Val His Ser Glu Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
20 25 30
Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe
35 40 45
Thr Glu Tyr Thr Ile His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu
50 55 60
Glu Trp Ile Gly Asn Ile Asn Pro Asn Asn Gly Gly Thr Thr Tyr Asn
65 70 75 80
Gln Lys Phe Glu Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Thr Asp
85 90 95
Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110
Tyr Tyr Cys Ala Ala Gly Trp Asn Phe Asp Tyr Trp Gly Gln Gly Thr
115 120 125
Leu Leu Thr Val Ser Ser
130

<210> 28
<211> 126
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J591

<400> 28
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15
Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Thr
20 25 30
Ser Val Gly Asp Arg Val Thr Leu Thr Cys Lys Ala Ser Gln Asp Val
35 40 45
Gly Thr Ala Val Asp Trp Tyr Gln Gln Lys Pro Gly Pro Ser Pro Lys
50 55 60
Leu Leu Ile Tyr Cys Ala Ser Thr Arg His Thr Gly Ile Pro Ser Arg
65 70 75 80
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser
85 90 95
Leu Gln Pro Glu Asp Phe Ala Asp Tyr Tyr Cys Gln Gln Tyr Asn Ser
100 105 110
Tyr Pro Leu Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
115 120 125

<210> 29
<211> 10
<212> PRT
<213> Mus musculus

<400> 29
Gly Phe Thr Phe Ser Asn Tyr Trp Met Asn
1 5 10

<210> 30
<211> 19
<212> PRT
<213> Mus musculus

<400> 30
Glu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala Glu Ser
1 5 10 15
Val Lys Gly

<210> 31
<211> 5
<212> PRT
<213> Mus musculus

<400> 31
Arg Trp Asn Asn Phe
1 5

<210> 32
<211> 11
<212> PRT
<213> Mus musculus

<400> 32
Lys Ala Ser Glu Asn Val Gly Thr Tyr Val Ser
1 5 10

<210> 33
<211> 7
<212> PRT
<213> Mus musculus

<400> 33
Gly Ala Ser Asn Arg Phe Thr
1 5

<210> 34
<211> 9
<212> PRT
<213> Mus musculus

<400> 34
Gly Gln Ser Tyr Thr Phe Pro Tyr Thr
1 5

<210> 35
<211> 82
<212> PRT
<213> Mus musculus

<400> 35
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Met Lys Leu Ser Cys Val Ala Ser Trp Val Arg Gln Ser Pro Glu
20 25 30
Lys Gly Leu Glu Trp Val Ala Arg Val Ile Ile Ser Arg Asp Asp Ser
35 40 45
Lys Ser Ser Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr
50 55 60
Gly Ile Tyr Tyr Cys Thr Arg Trp Gly Gln Gly Thr Thr Leu Thr Val
65 70 75 80
Ser Ser

<210> 36
<211> 80
<212> PRT
<213> Mus musculus

<400> 36
Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ile Ser Val Gly
1 5 10 15
Glu Arg Val Thr Leu Thr Cys Trp Tyr Gln Gln Lys Pro Glu Gln Ser
20 25 30
Pro Lys Met Leu Ile Tyr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly
35 40 45
Ser Ala Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Thr Glu Asp
50 55 60
Leu Val Asp Tyr Tyr Cys Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
65 70 75 80

<210> 37
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-4

<400> 37
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Met Lys Ile Ser Cys Val Ala Ser
20 25

<210> 38
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-4

<400> 38
Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val Ala
1 5 10

<210> 39
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-4

<400> 39
Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser Val Tyr Leu Gln
1 5 10 15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg
20 25 30

<210> 40
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-4

<400> 40
Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
1 5 10

<210> 41
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-5

<400> 41
Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
1 5 10 15
Glu Arg Met Thr Leu Thr Cys
20

<210> 42
<211> 15
<212> PRT
<213> Artificial Sequence

<220>

<223> deimmunized light chain J415-5

<400> 42
Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile Tyr
1 5 10 15

<210> 43
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-5

<400> 43
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ile
1 5 10 15
Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Val Asp Tyr Tyr Cys
20 25 30

<210> 44
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-5

<400> 44
Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
1 5 10

<210> 45
<211> 82
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-4

<400> 45
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Met Lys Ile Ser Cys Val Ala Ser Trp Val Arg Gln Ser Pro Glu
20 25 30
Lys Gly Leu Glu Trp Val Ala Arg Val Ile Ile Ser Arg Asp Asp Ser
35 40 45
Lys Ser Ser Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
50 55 60
Ala Val Tyr Tyr Cys Thr Arg Trp Gly Gln Gly Thr Thr Val Thr Val
65 70 75 80
Ser Ser

<210> 46
<211> 80
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-5

<400> 46
Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
1 5 10 15
Glu Arg Met Thr Leu Thr Cys Trp Tyr Gln Gln Lys Pro Thr Gln Ser
20 25 30
Pro Lys Met Leu Ile Tyr Gly Val Pro Asp Arg Phe Ser Gly Ser Gly
35 40 45
Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala Glu Asp
50 55 60
Leu Val Asp Tyr Tyr Cys Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
65 70 75 80

<210> 47
<211> 116
<212> PRT
<213> Mus musculus

<400> 47
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Met Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30
Trp Met Asn Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
35 40 45
Ala Glu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala Glu
50 55 60
Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser
65 70 75 80
Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
85 90 95
Tyr Cys Thr Arg Arg Trp Asn Asn Phe Trp Gly Gln Gly Thr Thr Leu
100 105 110
Thr Val Ser Ser
115

<210> 48
<211> 107
<212> PRT
<213> Mus musculus

<400> 48
Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ile Ser Val Gly
1 5 10 15
Glu Arg Val Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
20 25 30
Val Ser Trp Tyr Gln Gln Lys Pro Glu Gln Ser Pro Lys Met Leu Ile
35 40 45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60
Ser Gly Ser Ala Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Thr
65 70 75 80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
100 105

<210> 49
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-4

<400> 49
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Met Lys Ile Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30
Trp Met Asn Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
35 40 45
Ala Glu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala Glu
50 55 60
Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser
65 70 75 80
Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Thr Arg Arg Trp Asn Asn Phe Trp Gly Gln Gly Thr Thr Val
100 105 110
Thr Val Ser Ser
115

<210> 50
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-5

<400> 50
Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
1 5 10 15
Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
20 25 30
Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile
35 40 45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala
65 70 75 80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
100 105

<210> 51
<211> 348
<212> DNA
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-4

<400> 51
gaagtgaaac ttgaggagtc tggaggaggc ttggtgcaac ctggagggtc catgaaaatc 60
tcctgtgttg cctctggatt cactttcagt aattactgga tgaactgggt ccgccagtct 120
ccagagaagg ggcttgagtg ggttgctgaa attagatcgc aatctaataa ttttgcaaca 180
cattatgcgg agtctgtgaa agggagggtc atcatctcaa gagatgattc caagagtagt 240
gtctacctgc aaatgaacag tttgagagct gaagacactg ccgtttatta ctgtaccagg 300
cgatggaata atttctgggg ccaaggcacc actgtcacag tctcctca 348

<210> 52
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-5

<400> 52
aacattgtaa tgacccaatt tcccaaatcc atgtccgcct cagcaggaga gaggatgacc 60
ttgacctgca aggccagtga gaatgtgggt acttatgtgt cctggtatca acagaaacca 120
acacagtctc ctaagatgtt gatatacggg gcatccaacc ggttcactgg ggtcccagat 180
cgcttctccg gcagtggatc tggaacagat ttcattctga ccatcagcag tgtgcaggca 240
gaagaccttg tagattatta ctgtggacag agttacacct ttccgtacac gttcggaggg 300
gggaccaagc tggaaatgaa g 321

<210> 53
<211> 810
<212> DNA
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-1

<221> CDS
<222> (122)...(160)

<221> CDS
<222> (249)...(608)

<400> 53
aagcttatga atatgcaaat cctctgaatc tacatggtaa atataggttt gtctatacca 60
caaacagaaa aacatgagat cacagttctc tctacagtta ctgagcacac aggacctcac 120
c atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gctacaggta 170
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr
1 5 10

aggggctcac agtagcaggc ttgaggtctg gacatatata tgggtgacaa tgacatccac 230
tttgcctttc tctccaca ggt gtc cac tcc gaa gtg aaa ctt gag gag tct 281
Gly Val His Ser Glu Val Lys Leu Glu Glu Ser
15 20

gga gga ggc ttg gtg caa cct gga ggg tcc atg aaa atc tcc tgt aaa 329
Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Met Lys Ile Ser Cys Lys
25 30 35 40

gcc tct gga ttc act ttc agt aat tac tgg atg aac tgg gtc cgc cag 377
Ala Ser Gly Phe Thr Phe Ser Asn Tyr Trp Met Asn Trp Val Arg Gln
45 50 55

act cca gag aag ggg ctt gag tgg gtt gct ctt att aga tcg caa tct 425
Thr Pro Glu Lys Gly Leu Glu Trp Val Ala Leu Ile Arg Ser Gln Ser
60 65 70

aat aat ttt gca aca cat tat gcg gag tct gtg aaa ggg agg gtc atc 473
Asn Asn Phe Ala Thr His Tyr Ala Glu Ser Val Lys Gly Arg Val Ile
75 80 85

atc tca aga gat gat tcc aag agt agt gtc tac ctg caa atg aac agt 521
Ile Ser Arg Asp Asp Ser Lys Ser Ser Val Tyr Leu Gln Met Asn Ser

```
    90                  95                 100

ttg aga gct gaa gac act gcc gtt tat tac tgt acc agg cga tgg aat      569
Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg Arg Trp Asn
105                 110                 115                 120

aat ttc tgg ggc caa ggc acc act gtc aca gtc tcc tca ggtgagtcct      618
Asn Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
                125                 130

tacaacctct ctcttctatt cagcttaaat agattttact gcatttgttg gggggaaat    678
gtgtgtatct gaatttcagg tcatgaagga ctagggacac cttgggagtc agaaagggtc    738
attgggagcc cgggctgatg cagacagaca tcctcagctc ccagacttca tggccagaga    798
tttataggat cc                                                       810

<210> 54
<211> 133
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-1

<400> 54
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Gly Val His
 1               5                  10                  15
Ser Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
            20                  25                  30
Gly Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Phe Thr Phe Ser Asn
        35                  40                  45
Tyr Trp Met Asn Trp Val Arg Gln Thr Pro Glu Lys Gly Leu Glu Trp
    50                  55                  60
Val Ala Leu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala
65                  70                  75                  80
Glu Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser
                85                  90                  95
Ser Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
            100                 105                 110
Tyr Tyr Cys Thr Arg Arg Trp Asn Asn Phe Trp Gly Gln Gly Thr Thr
        115                 120                 125
Val Thr Val Ser Ser
    130

<210> 55
<211> 810
<212> DNA
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-1

<400> 55
ggatcctata aatctctggc catgaagtct gggagctgag gatgtctgtc tgcatcagcc     60
cgggctccca atgacccttt ctgactccca aggtgtccct agtccttcat gacctgaaat    120
tcagatacac acatttcccc cccaacaaat gcagtaaaat ctatttaagc tgaatagaag    180
agagaggttg taaggactca cctgaggaga ctgtgacagt ggtgccttgg ccccagaaat    240
tattccatcg cctggtacag taataaacgg cagtgtcttc agctctcaaa ctgttcattt    300
gcaggtagac actactcttg gaatcatctc ttgagatgat gaccctccct ttcacagact    360
ccgcataatg tgttgcaaaa ttattagatt gcgatctaat aagagcaacc cactcaagcc    420
ccttctctgg agtctggcgg acccagttca tccagtaatt actgaaagtg aatccagagg    480
ctttacagga gattttcatg gaccctccag gttgcaccaa gcctcctcca gactcctcaa    540
```

```
gtttcacttc ggagtggaca cctgtggaga gaaaggcaaa gtggatgtca ttgtcaccca      600
tatatatgtc cagacctcaa gcctgctact gtgagcccct tacctgtagc tgttgctacc      660
aagaagagga tgatacagct ccatcccatg gtgaggtcct gtgtgctcag taactgtaga      720
gagaactgtg atctcatgtt tttctgtttg tggtatagac aaacctatat ttaccatgta      780
gattcagagg atttgcatat tcataagctt                                       810

<210> 56
<211> 620
<212> DNA
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-1

<221> CDS
<222> (122)...(160)

<221> CDS
<222> (249)...(581)

<400> 56
aagcttatga atatgcaaat cctctgaatc tacatggtaa atataggttt gtctatacca       60
caaacagaaa aacatgagat cacagttctc tctacagtta ctgagcacac aggacctcac      120
c atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gctacaggta       170
  Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr
   1               5                  10

aggggctcac agtagcaggc ttgaggtctg gacatatata tgggtgacaa tgacatccac      230
tttgcctttc tctccaca ggt gtc cac tcc aac att gta atg acc caa tcc         281
                    Gly Val His Ser Asn Ile Val Met Thr Gln Ser
                         15                  20

ccc aaa tcc atg tcc gcc tca gca gga gag agg atg acc ttg acc tgc        329
Pro Lys Ser Met Ser Ala Ser Ala Gly Glu Arg Met Thr Leu Thr Cys
 25                  30                  35                  40

aag gcc agt gag aat tcc ggt act tat gtg tcc tgg tat caa cag aaa        377
Lys Ala Ser Glu Asn Ser Gly Thr Tyr Val Ser Trp Tyr Gln Gln Lys
                45                  50                  55

cca aca cag tct cct aag atg ttg ata tac ggg gca tcc aac cgg ttc        425
Pro Thr Gln Ser Pro Lys Met Leu Ile Tyr Gly Ala Ser Asn Arg Phe
            60                  65                  70

act ggg gtc cca gat cgc ttc tcc ggc agt gga tct gga aca gat ttc        473
Thr Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
        75                  80                  85

att ctg acc gcc agc agt gtg cag gca gaa gac cct gta gat tat tac        521
Ile Leu Thr Ala Ser Ser Val Gln Ala Glu Asp Pro Val Asp Tyr Tyr
    90                  95                  100

tgt gga cag agt tac acc ttt ccg tac acg ttc gga ggg ggg acc aag        569
Cys Gly Gln Ser Tyr Thr Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys
105                 110                 115                 120

ctg gaa atg aag cgtgagtaga atttaaactt tgcttcctca gttggatcc             620
Leu Glu Met Lys
```

<210> 57
<211> 124
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-1

<400> 57
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Gly Val His
1 5 10 15
Ser Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser Ala Ser Ala
20 25 30
Gly Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Ser Gly Thr
35 40 45
Tyr Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu
50 55 60
Ile Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser
65 70 75 80
Gly Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ala Ser Ser Val Gln
85 90 95
Ala Glu Asp Pro Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro
100 105 110
Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
115 120

<210> 58
<211> 620
<212> DNA
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-1

<400> 58
ggatccaact gaggaagcaa agtttaaatt ctactcacgc ttcatttcca gcttggtccc 60
ccctccgaac gtgtacggaa aggtgtaact ctgtccacag taataatcta cagggtcttc 120
tgcctgcaca ctgctggcgg tcagaatgaa atctgttcca gatccactgc cggagaagcg 180
atctgggacc ccagtgaacc ggttggatgc cccgtatatc aacatcttag gagactgtgt 240
tggtttctgt tgataccagg acacataagt accggaattc tcactggcct tgcaggtcaa 300
ggtcatcctc tctcctgctg aggcggacat ggatttgggg gattgggtca ttacaatgtt 360
ggagtggaca cctgtggaga gaaaggcaaa gtggatgtca ttgtcaccca tatatatgtc 420
cagacctcaa gcctgctact gtgagcccct tacctgtagc tgttgctacc aagaagagga 480
tgatacagct ccatcccatg gtgaggtcct gtgtgctcag taactgtaga gagaactgtg 540
atctcatgtt tttctgtttg tggtatagac aaacctatat ttaccatgta gattcagagg 600
atttgcatat tcataagctt 620

<210> 59
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-2

<400> 59
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Met Lys Ile Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30
Trp Met Asn Trp Val Arg Gln Thr Pro Glu Lys Gly Leu Glu Trp Val

```
        35                  40                  45
Ala Leu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala Glu
    50                  55                  60
Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser
65                  70                  75                  80
Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Thr Arg Arg Trp Asn Asn Phe Trp Gly Gln Gly Thr Thr Val
            100                 105                 110
Thr Val Ser Ser
        115
```

<210> 60
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-3

<400> 60

```
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1               5                  10                  15
Ser Met Lys Ile Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20                  25                  30
Trp Met Asn Trp Val Arg Gln Thr Pro Glu Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Glu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala Glu
    50                  55                  60
Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser
65                  70                  75                  80
Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Thr Arg Arg Trp Asn Asn Phe Trp Gly Gln Gly Thr Thr Val
            100                 105                 110
Thr Val Ser Ser
        115
```

<210> 61
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> majority sequence

<400> 61

```
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1               5                  10                  15
Ser Met Lys Ile Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20                  25                  30
Trp Met Asn Trp Val Arg Gln Thr Pro Glu Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Glu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala Glu
    50                  55                  60
Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser
65                  70                  75                  80
Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Thr Arg Arg Trp Asn Asn Phe Trp Gly Gln Gly Thr Thr Val
            100                 105                 110
```

Thr Val Ser Ser
115

<210> 62
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-2

<400> 62
Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser Ala Ser Ala Gly
1 5 10 15
Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
20 25 30
Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile
35 40 45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ala Ser Ser Val Gln Ala
65 70 75 80
Glu Asp Pro Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
100 105

<210> 63
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-3

<400> 63
Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser Ala Ser Ala Gly
1 5 10 15
Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
20 25 30
Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile
35 40 45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ala Ser Ser Val Gln Ala
65 70 75 80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
100 105

<210> 64
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-4

<400> 64
Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser Ala Ser Ala Gly

```
 1               5                  10                  15
Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
            20                  25                  30
Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile
        35                  40                  45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala
65                  70                  75                  80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
            100                 105
```

<210> 65
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-6

<400> 65

```
Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
 1               5                  10                  15
Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
            20                  25                  30
Val Ser Trp Tyr Gln Gln Lys Pro Glu Gln Ser Pro Lys Met Leu Ile
        35                  40                  45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala
65                  70                  75                  80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
            100                 105
```

<210> 66
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-7

<400> 66

```
Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
 1               5                  10                  15
Glu Arg Val Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
            20                  25                  30
Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile
        35                  40                  45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala
65                  70                  75                  80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
            100                 105
```

<210> 67
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-8

<400> 67
Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
1 5 10 15
Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Ser Gly Thr Tyr
20 25 30
Val Ser Trp Tyr Gln Gln Lys Pro Glu Gln Ser Pro Lys Met Leu Ile
35 40 45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala
65 70 75 80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
100 105

<210> 68
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> majority sequence

<400> 68
Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
1 5 10 15
Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
20 25 30
Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile
35 40 45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala
65 70 75 80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
100 105

<210> 69
<211> 123
<212> PRT
<213> Mus musculus

<400> 69
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Met Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30
Trp Met Asn Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
35 40 45

Ala Glu Ile Arg Leu Lys Ser Asp Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ser
65 70 75 80
Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
85 90 95
Tyr Cys Thr Thr Gly Gly Tyr Gly Gly Arg Arg Ser Trp Phe Ala Tyr
100 105 110
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 70
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> *majority sequence*

<400> 70
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Met Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30
Trp Met Asn Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
35 40 45
Ala Glu Ile Arg Leu Gln Ser Asp Asn Phe Ala Thr His Tyr Ala Glu
50 55 60
Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser
65 70 75 80
Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
85 90 95
Tyr Cys Thr Thr Gly Gly Tyr Gly Gly Arg Arg Ser Trp Asn Ala Phe
100 105 110
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 71
<211> 113
<212> PRT
<213> Mus musculus

<400> 71
Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15
Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30
Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45
Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80
Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
85 90 95
Asp Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
100 105 110
Lys

<210> 72

<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> majority sequence

<400> 72
Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
 1   5   10   15
Glu Lys Val Thr Leu Ser Cys Lys Ala Ser Glu Ser Leu Leu Asn Val
            20   25   30
Gly Asn Gln Lys Thr Tyr Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35   40   45
Ser Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val
    50   55   60
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr
65   70   75   80
Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gly Asn
                85   90   95
Ser Tyr Ser Phe Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu
            100   105   110
Lys

<210> 73
<211> 354
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(354)

<400> 73
gag gtc cag ctg cag cag tct gga cct gag ctg gtt aag cct ggg gct 48
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1   5   10   15

tca gtg aag atg tcc tgc aag gct tct gga tac aca ttc act ggc tat 96
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20   25   30

gtt atg cac tgg gtg aag cag aag cct gga cag gtc ctt gag tgg att 144
Val Met His Trp Val Lys Gln Lys Pro Gly Gln Val Leu Glu Trp Ile
        35   40   45

gga tat att aat cct tac aat gat gtt act agg tat aat ggg aag ttc 192
Gly Tyr Ile Asn Pro Tyr Asn Asp Val Thr Arg Tyr Asn Gly Lys Phe
    50   55   60

aaa ggc aag gcc aca ctg acc tca gac aaa tat tcc agc aca gcc tac 240
Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Tyr Ser Ser Thr Ala Tyr
65   70   75   80

atg gag ctc agc ggc ctg acc tct gag gac tct gcg gtc tat tac tgt 288
Met Glu Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85   90   95

gca aga ggg gag aac tgg tac tac ttt gac tcc tgg ggc cga ggc gcc 336
Ala Arg Gly Glu Asn Trp Tyr Tyr Phe Asp Ser Trp Gly Arg Gly Ala

100 105 110

act ctc aca gtc tcc tca 354
Thr Leu Thr Val Ser Ser
115

<210> 74
<211> 118
<212> PRT
<213> Mus musculus

<400> 74
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
20 25 30
Val Met His Trp Val Lys Gln Lys Pro Gly Gln Val Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Tyr Asn Asp Val Thr Arg Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Tyr Ser Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Glu Asn Trp Tyr Tyr Phe Asp Ser Trp Gly Arg Gly Ala
100 105 110
Thr Leu Thr Val Ser Ser
115

<210> 75
<211> 354
<212> DNA
<213> Mus musculus

<400> 75
tgaggagact gtgagagtgg cgcctcggcc ccaggagtca aagtagtacc agttctcccc 60
tcttgcacag taatagaccg cagagtcctc agaggtcagg ccgctgagct ccatgtaggc 120
tgtgctggaa tatttgtctg aggtcagtgt ggccttgcct ttgaacttcc cattatacct 180
agtaacatca ttgtaaggat taatatatcc aatccactca aggacctgtc caggcttctg 240
cttcacccag tgcataacat agccagtgaa tgtgtatcca gaagccttgc aggacatctt 300
cactgaagcc ccaggcttaa ccagctcagg tccagactgc tgcagctgga cctc 354

<210> 76
<211> 333
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(333)

<400> 76
gac att gtg ctg acc caa tct cca gct tct ttg gct gtg tct cta gga 48
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

cag agg gcc acc ata tcc tgc aga gcc agt gaa agt att gat agt tat 96
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Ile Asp Ser Tyr
20 25 30

```
gac aat act ttt atg cac tgg tac cag cag aaa cca gga cag cca ccc      144
Asp Asn Thr Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

aac ctc ctc atc ttt cgt gca tcc atc cta gaa tct ggg atc cct gcc      192
Asn Leu Leu Ile Phe Arg Ala Ser Ile Leu Glu Ser Gly Ile Pro Ala
    50                  55                  60

agg ttc agt ggc agt ggg tct ggg aca gac ttc acc ctc acc att tat      240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Tyr
65                  70                  75                  80

cct gtg gag gct gat gat gtt gca acc tat tac tgt cac caa agt att      288
Pro Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys His Gln Ser Ile
                85                  90                  95

gag gat ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa          333
Glu Asp Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
```

<210> 77
<211> 111
<212> PRT
<213> Mus musculus

<400> 77

```
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1               5                  10                  15
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Ile Asp Ser Tyr
            20                  25                  30
Asp Asn Thr Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45
Asn Leu Leu Ile Phe Arg Ala Ser Ile Leu Glu Ser Gly Ile Pro Ala
    50                  55                  60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Tyr
65                  70                  75                  80
Pro Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys His Gln Ser Ile
                85                  90                  95
Glu Asp Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
```

<210> 78
<211> 333
<212> DNA
<213> Mus musculus

<400> 78

```
ttttatttcc agcttggtcc cccctccgaa cgtgtacgga tcctcaatac tttggtgaca      60
gtaataggtt gcaacatcat cagcctccac aggataaatg gtgagggtga agtctgtccc     120
agacccactg ccactgaacc tggcagggat cccagattct aggatggatg cacgaaagat     180
gaggaggttg ggtggctgtc ctggtttctg ctggtaccag tgcataaaag tattgtcata     240
actatcaata ctttcactgg ctctgcagga tatggtggcc ctctgtccta gagacacagc     300
caaagaagct ggagattggg tcagcacaat gtc                                  333
```

<210> 79
<211> 125
<212> PRT
<213> Mus musculus

<400> 79

```
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1               5                  10                  15
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20                  25                  30
Tyr Met Asn Asn Trp Val Lys Gln Ser Pro Gly Lys Ser Leu Glu Trp
        35                  40                  45
Ile Gly Asp Ile Asn Pro Gly Asn Gly Gly Thr Ser Tyr Asn Gln Lys
    50                  55                  60
Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala
65                  70                  75                  80
Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
                85                  90                  95
Cys Ala Arg Gly Tyr Tyr Ser Ser Ser Tyr Met Ala Tyr Tyr Ala Phe
            100                 105                 110
Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120                 125
```

<210> 80
<211> 125
<212> PRT
<213> Artificial Sequence

<220>
<223> majority sequence

<400> 80

```
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1               5                  10                  15
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30
Val Met Asn Asn Trp Val Lys Gln Ser Pro Gly Gln Val Leu Glu Trp
        35                  40                  45
Ile Gly Asp Ile Asn Pro Gly Asn Gly Gly Thr Ser Tyr Asn Gly Lys
    50                  55                  60
Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala
65                  70                  75                  80
Tyr Met Glu Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
                85                  90                  95
Cys Ala Arg Gly Glu Asn Ser Ser Ser Tyr Met Ala Tyr Tyr Ala Phe
            100                 105                 110
Asp Ser Trp Gly Gln Gly Ala Thr Val Thr Val Ser Ser
        115                 120                 125
```

<210> 81
<211> 112
<212> PRT
<213> Mus musculus

<400> 81

```
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1               5                  10                  15
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr
            20                  25                  30
Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45
Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
    50                  55                  60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65                  70                  75                  80
Pro Val Glu Glu Asp Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Asn
                85                  90                  95
```

```
Glu Asp Pro Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

<210> 82
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> majority sequence

<400> 82
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1               5                  10                  15
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr
            20                  25                  30
Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45
Asn Leu Leu Ile Phe Ala Ala Ser Ile Leu Glu Ser Gly Val Pro Ala
    50                  55                  60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile His
65                  70                  75                  80
Pro Val Glu Ala Asp Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Ile
                85                  90                  95
Glu Asp Pro Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

<210> 83
<211> 363
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(363)

<400> 83
cag gtg cag cta aag gag tca gga cct ggc ctg gtg gcg tcc tca cag       48
Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Ser Ser Gln
 1               5                  10                  15

agc ctg tcc atc aca tgc acc gtc tca gga ttc tca tta acc gcc tat       96
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr
            20                  25                  30

ggt att aac tgg gtt cgc cag cct cca gga aag ggt ctg gag tgg ctg      144
Gly Ile Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45

gga gtg ata tgg cct gat gga aac aca gac tat aat tca act ctc aaa      192
Gly Val Ile Trp Pro Asp Gly Asn Thr Asp Tyr Asn Ser Thr Leu Lys
    50                  55                  60

tcc aga ctg aac atc ttc aag gac aac tcc aag aac caa gtt ttc tta      240
Ser Arg Leu Asn Ile Phe Lys Asp Asn Ser Lys Asn Gln Val Phe Leu
65                  70                  75                  80

aaa atg agc agt ttc caa act gat gac aca gcc aga tac ttc tgt gcc      288
Lys Met Ser Ser Phe Gln Thr Asp Asp Thr Ala Arg Tyr Phe Cys Ala
                85                  90                  95
```

```
aga gat tcg tat ggt aac ttc aag agg ggt tgg ttt gac ttc tgg ggc      336
Arg Asp Ser Tyr Gly Asn Phe Lys Arg Gly Trp Phe Asp Phe Trp Gly
            100                 105                 110

cag ggc acc act ctc aca gtc tcc tca                                  363
Gln Gly Thr Thr Leu Thr Val Ser Ser
        115                 120
```

<210> 84
<211> 121
<212> PRT
<213> Mus musculus

<400> 84

```
Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Ser Ser Gln
 1               5                  10                  15
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr
            20                  25                  30
Gly Ile Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45
Gly Val Ile Trp Pro Asp Gly Asn Thr Asp Tyr Asn Ser Thr Leu Lys
    50                  55                  60
Ser Arg Leu Asn Ile Phe Lys Asp Asn Ser Lys Asn Gln Val Phe Leu
65                  70                  75                  80
Lys Met Ser Ser Phe Gln Thr Asp Asp Thr Ala Arg Tyr Phe Cys Ala
                85                  90                  95
Arg Asp Ser Tyr Gly Asn Phe Lys Arg Gly Trp Phe Asp Phe Trp Gly
            100                 105                 110
Gln Gly Thr Thr Leu Thr Val Ser Ser
        115                 120
```

<210> 85
<211> 363
<212> DNA
<213> Mus musculus

<400> 85

```
tgaggagact gtgagagtgg tgccctggcc ccagaagtca aaccaacccc tcttgaagtt      60
accatacgaa tctctggcac agaagtatct ggctgtgtca tcagtttgga aactgctcat     120
ttttaagaaa acttggttct tggagttgtc cttgaagatg ttcagtctgg atttgagagt     180
tgaattatag tctgtgtttc catcaggcca tatcactccc agccactcca gacccttcc      240
tggaggctgg cgaacccagt taataccata ggcggttaat gagaatcctg agacggtgca     300
tgtgatggac aggctctgtg aggacgccac caggccaggt cctgactcct ttagctgcac     360
ctg                                                                   363
```

<210> 86
<211> 321
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(321)

<400> 86

```
aac att gtg atg acc cag tct caa aaa ttc atg tcc aca tca cca gga       48
Asn Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Pro Gly
 1               5                  10                  15

gac agg gtc agg gtc acc tgc aag gcc agt cag aat gtg ggt tct gat       96
```

```
Asp Arg Val Arg Val Thr Cys Lys Ala Ser Gln Asn Val Gly Ser Asp
             20                  25                  30

gta gcc tgg tat caa gcg aaa cca gga caa tct cct aga ata ctg att      144
Val Ala Trp Tyr Gln Ala Lys Pro Gly Gln Ser Pro Arg Ile Leu Ile
         35                  40                  45

tac tcg aca tcc tac cgt tac agt ggg gtc cct gat cgc ttc aca gcc      192
Tyr Ser Thr Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr Ala
     50                  55                  60

tat gga tct ggg aca gat ttc act ctc acc att acc aat gtg cag tct      240
Tyr Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
 65                  70                  75                  80

gaa gac ttg aca gag tat ttc tgt cag caa tat aat agc tat cct ctc      288
Glu Asp Leu Thr Glu Tyr Phe Cys Gln Gln Tyr Asn Ser Tyr Pro Leu
                 85                  90                  95

acg ttc ggt gct ggg acc aag ctg gag ctg aaa                          321
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
            100                 105


<210> 87
<211> 107
<212> PRT
<213> Mus musculus

<400> 87
Asn Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Pro Gly
 1               5                  10                  15
Asp Arg Val Arg Val Thr Cys Lys Ala Ser Gln Asn Val Gly Ser Asp
            20                  25                  30
Val Ala Trp Tyr Gln Ala Lys Pro Gly Gln Ser Pro Arg Ile Leu Ile
        35                  40                  45
Tyr Ser Thr Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr Ala
    50                  55                  60
Tyr Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
65                  70                  75                  80
Glu Asp Leu Thr Glu Tyr Phe Cys Gln Gln Tyr Asn Ser Tyr Pro Leu
                85                  90                  95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
            100                 105

<210> 88
<211> 321
<212> DNA
<213> Mus musculus

<400> 88
tttcagctcc agcttggtcc cagcaccgaa cgtgagagga tagctattat attgctgaca      60
gaaatactct gtcaagtctt cagactgcac attggtaatg gtgagagtga aatctgtccc     120
agatccatag gctgtgaagc gatcagggac cccactgtaa cggtaggatg tcgagtaaat     180
cagtattcta ggagattgtc ctggtttcgc ttgataccag gctacatcag aacccacatt     240
ctgactggcc ttgcaggtga ccctgaccct gtctcctggt gatgtggaca tgaatttttg     300
agactgggtc atcacaatgt t                                               321

<210> 89
<211> 121
<212> PRT
```

<213> Mus musculus

<400> 89
Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Ser Ser Gln
1 5 10 15
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr
20 25 30
Gly Ile Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Pro Asp Gly Asn Thr Asp Tyr Asn Ser Thr Leu Lys
50 55 60
Ser Arg Leu Asn Ile Phe Lys Asp Asn Ser Lys Asn Gln Val Phe Leu
65 70 75 80
Lys Met Ser Ser Phe Gln Thr Asp Asp Thr Ala Arg Tyr Phe Cys Ala
85 90 95
Arg Asp Ser Tyr Gly Asn Phe Lys Arg Gly Trp Phe Asp Phe Trp Gly
100 105 110
Gln Gly Thr Thr Leu Thr Val Ser Ser
115 120

<210> 90
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> majority sequence

<400> 90
Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Ser Ser Gln
1 5 10 15
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr
20 25 30
Gly Ile Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Pro Asp Gly Asn Thr Asp Tyr Asn Ser Thr Leu Lys
50 55 60
Ser Arg Leu Asn Ile Phe Lys Asp Asn Ser Lys Asn Gln Val Phe Leu
65 70 75 80
Lys Met Ser Ser Phe Gln Thr Asp Asp Thr Ala Arg Tyr Phe Cys Ala
85 90 95
Arg Asp Ser Tyr Gly Asn Phe Lys Arg Gly Trp Phe Asp Phe Trp Gly
100 105 110
Gln Gly Thr Thr Leu Thr Val Ser Ser
115 120

<210> 91
<211> 113
<212> PRT
<213> Mus musculus

<400> 91
Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15
Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30
Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45
Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr

65 70 75 80
Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
85 90 95
Asp Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
100 105 110
Lys

<210> 92
<211> 115
<212> PRT
<213> Artificial Sequence

<220>
<223> majority sequence

<400> 92
Asp Ile Val Met Thr Gln Ser Gln Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15
Asp Lys Val Thr Val Ser Cys Lys Ala Ser Gln Ser Leu Leu Asn Val
20 25 30
Gly Ser Asp Lys Asn Tyr Val Ala Trp Tyr Gln Ala Lys Pro Gly Gln
35 40 45
Ser Pro Lys Leu Leu Ile Tyr Ser Ala Ser Thr Arg Glu Ser Gly Val
50 55 60
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80
Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Asn
85 90 95
Asp Asn Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
100 105 110
Lys Arg Ala
115

<210> 93
<211> 10
<212> PRT
<213> Mus musculus

<400> 93
Gly Tyr Thr Phe Thr Gly Tyr Val Met His
1 5 10

<210> 94
<211> 17
<212> PRT
<213> Mus musculus

<400> 94
Tyr Ile Asn Pro Tyr Asn Asp Val Thr Arg Tyr Asn Gly Lys Phe Lys
1 5 10 15
Gly

<210> 95
<211> 9
<212> PRT
<213> Mus musculus

<400> 95
Gly Glu Asn Trp Tyr Tyr Phe Asp Ser

1 5

<210> 96
<211> 15
<212> PRT
<213> Mus musculus

<400> 96
Arg Ala Ser Glu Ser Ile Asp Ser Tyr Asp Asn Thr Phe Met His
1 5 10 15

<210> 97
<211> 7
<212> PRT
<213> Mus Musculus

<400> 97
Arg Ala Ser Ile Leu Glu Ser
1 5

<210> 98
<211> 9
<212> PRT
<213> Mus musculus

<400> 98
His Gln Ser Ile Glu Asp Pro Tyr Thr
1 5

<210> 99
<211> 10
<212> PRT
<213> Mus musculus

<400> 99
Gly Phe Ser Leu Thr Ala Tyr Gly Ile Asn
1 5 10

<210> 100
<211> 16
<212> PRT
<213> Mus musculus

<400> 100
Val Ile Trp Pro Asp Gly Asn Thr Asp Tyr Asn Ser Thr Leu Lys Ser
1 5 10 15

<210> 101
<211> 13
<212> PRT
<213> Mus musculus

<400> 101
Asp Ser Tyr Gly Asn Phe Lys Arg Gly Trp Phe Asp Phe
1 5 10

<210> 102
<211> 11
<212> PRT
<213> Mus musculus

<400> 102
Lys Ala Ser Gln Asn Val Gly Ser Asp Val Ala
1 5 10

<210> 103
<211> 7
<212> PRT
<213> Mus musculus

<400> 103
Ser Thr Ser Tyr Arg Tyr Ser
1 5

<210> 104
<211> 9
<212> PRT
<213> Mus musculus

<400> 104
Gln Gln Tyr Asn Ser Tyr Pro Leu Thr
1 5

<210> 105
<211> 25
<212> PRT
<213> Mus musculus

<400> 105
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser
20 25

<210> 106
<211> 14
<212> PRT
<213> Mus musculus

<400> 106
Trp Val Lys Gln Lys Pro Gly Gln Val Leu Glu Trp Ile Gly
1 5 10

<210> 107
<211> 32
<212> PRT
<213> Mus musculus

<400> 107
Lys Ala Thr Leu Thr Ser Asp Lys Tyr Ser Ser Thr Ala Tyr Met Glu
1 5 10 15
Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 108
<211> 11
<212> PRT
<213> Mus musculus

<400> 108
Trp Gly Arg Gly Ala Thr Leu Thr Val Ser Ser
1 5 10

<210> 109
<211> 82
<212> PRT
<213> Mus musculus

<400> 109
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser Trp Val Lys Gln Lys Pro Gly
20 25 30
Gln Val Leu Glu Trp Ile Gly Lys Ala Thr Leu Thr Ser Asp Lys Tyr
35 40 45
Ser Ser Thr Ala Tyr Met Glu Leu Ser Gly Leu Thr Ser Glu Asp Ser
50 55 60
Ala Val Tyr Tyr Cys Ala Arg Trp Gly Arg Gly Ala Thr Leu Thr Val
65 70 75 80
Ser Ser

<210> 110
<211> 23
<212> PRT
<213> Mus musculus

<400> 110
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15
Gln Arg Ala Thr Ile Ser Cys
20

<210> 111
<211> 15
<212> PRT
<213> Mus musculus

<400> 111
Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Asn Leu Leu Ile Phe
1 5 10 15

<210> 112
<211> 32
<212> PRT
<213> Mus musculus

<400> 112
Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15
Leu Thr Ile Tyr Pro Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys
20 25 30

<210> 113
<211> 10
<212> PRT
<213> Mus musculus

<400> 113
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 114

<211> 80
<212> PRT
<213> Mus musculus

<400> 114
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15
Gln Arg Ala Thr Ile Ser Cys Trp Tyr Gln Gln Lys Pro Gly Gln Pro
20 25 30
Pro Asn Leu Leu Ile Phe Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly
35 40 45
Ser Gly Thr Asp Phe Thr Leu Thr Ile Tyr Pro Val Glu Ala Asp Asp
50 55 60
Val Ala Thr Tyr Tyr Cys Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
65 70 75 80

<210> 115
<211> 25
<212> PRT
<213> Mus musculus

<400> 115
Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Ser Ser Gln
1 5 10 15
Ser Leu Ser Ile Thr Cys Thr Val Ser
20 25

<210> 116
<211> 14
<212> PRT
<213> Mus musculus

<400> 116
Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu Gly
1 5 10

<210> 117
<211> 32
<212> PRT
<213> Mus musculus

<400> 117
Arg Leu Asn Ile Phe Lys Asp Asn Ser Lys Asn Gln Val Phe Leu Lys
1 5 10 15
Met Ser Ser Phe Gln Thr Asp Asp Thr Ala Arg Tyr Phe Cys Ala Arg
20 25 30

<210> 118
<211> 11
<212> PRT
<213> Mus musculus

<400> 118
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
1 5 10

<210> 119
<211> 82
<212> PRT
<213> Mus musculus

<400> 119
Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Ser Ser Gln
1 5 10 15
Ser Leu Ser Ile Thr Cys Thr Val Ser Trp Val Arg Gln Pro Pro Gly
20 25 30
Lys Gly Leu Glu Trp Leu Gly Arg Leu Asn Ile Phe Lys Asp Asn Ser
35 40 45
Lys Asn Gln Val Phe Leu Lys Met Ser Ser Phe Gln Thr Asp Asp Thr
50 55 60
Ala Arg Tyr Phe Cys Ala Arg Trp Gly Gln Gly Thr Thr Leu Thr Val
65 70 75 80
Ser Ser

<210> 120
<211> 23
<212> PRT
<213> Mus musculus

<400> 120
Asn Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Pro Gly
1 5 10 15
Asp Arg Val Arg Val Thr Cys
20

<210> 121
<211> 15
<212> PRT
<213> Mus musculus

<400> 121
Trp Tyr Gln Ala Lys Pro Gly Gln Ser Pro Arg Ile Leu Ile Tyr
1 5 10 15

<210> 122
<211> 32
<212> PRT
<213> Mus musculus

<400> 122
Gly Val Pro Asp Arg Phe Thr Ala Tyr Gly Ser Gly Thr Asp Phe Thr
1 5 10 15
Leu Thr Ile Thr Asn Val Gln Ser Glu Asp Leu Thr Glu Tyr Phe Cys
20 25 30

<210> 123
<211> 10
<212> PRT
<213> Mus musculus

<400> 123
Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
1 5 10

<210> 124
<211> 80
<212> PRT
<213> Mus musculus

<400> 124
Asn Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Pro Gly

```
 1               5                  10                  15
Asp Arg Val Arg Val Thr Cys Trp Tyr Gln Ala Lys Pro Gly Gln Ser
            20                  25                  30
Pro Arg Ile Leu Ile Tyr Gly Val Pro Asp Arg Phe Thr Ala Tyr Gly
        35                  40                  45
Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser Glu Asp
    50                  55                  60
Leu Thr Glu Tyr Phe Cys Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
65                  70                  75                  80
```

<210> 125
<211> 348
<212> DNA
<213> Mus musculus

<400> 125
gaagtgaagc ttgaggagtc tggaggaggc ttggtgcaac ctggaggatc catgaaactc 60
tcctgtgttg cctctggatt cactttcagt aattactgga tgaactgggt ccgccagtct 120
ccagagaagg ggcttgagtg ggttgctgaa attagatcgc aatctaataa ttttgcaaca 180
cattatgcgg agtctgtgaa agggagggtc atcatctcaa gagatgattc caagagtagt 240
gtctacctgc aaatgaacaa cttgagagct gaagacactg gcatttatta ctgtaccagg 300
cgatggaata atttctgggg ccaaggcacc actctcacag tctcctca 348

<210> 126
<211> 348
<212> DNA
<213> Mus musculus

<400> 126
tgaggagact gtgagagtgg tgccttggcc ccagaaatta ttccatcgcc tggtacagta 60
ataaatgcca gtgtcttcag ctctcaagtt gttcatttgc aggtagacac tactcttgga 120
atcatctctt gagatgatga ccctcccttt cacagactcc gcataatgtg ttgcaaaatt 180
attagattgc gatctaattt cagcaaccca ctcaagcccc ttctctggag actggcggac 240
ccagttcatc cagtaattac tgaaagtgaa tccagaggca acacaggaga gtttcatgga 300
tcctccaggt tgcaccaagc ctcctccaga ctcctcaagc ttcacttc 348

<210> 127
<211> 321
<212> DNA
<213> Mus musculus

<400> 127
aacattgtaa tgacccaatt tcccaaatcc atgtccattt cagtaggaga gagggtcacc 60
ttgacctgca aggccagtga gaatgtgggt acttatgtgt cctggtatca acagaaacca 120
gaacagtctc ctaagatgtt gatatacggg gcatccaacc ggttcactgg ggtccccgat 180
cgcttcacag gcagtggatc tgcaacagat ttcattctga ccatcagcag tgtgcagact 240
gaagaccttg tagattatta ctgtggacag agttacacct ttccgtacac gttcggaggg 300
gggaccaagc tggaaatgaa g 321

<210> 128
<211> 321
<212> DNA
<213> Mus musculus

<400> 128
cttcatttcc agcttggtcc cccctccgaa cgtgtacgga aaggtgtaac tctgtccaca 60
gtaataatct acaaggtctt cagtctgcac actgctgatg gtcagaatga aatctgttgc 120
agatccactg cctgtgaagc gatcggggac cccagtgaac cggttggatg ccccgtatat 180
caacatctta ggagactgtt ctggtttctg ttgataccag gacacataag tacccacatt 240
ctcactggcc ttgcaggtca aggtgaccct ctctcctact gaaatggaca tggatttggg 300
aaattgggtc attacaatgt t 321

American Type Culture Collection

12301 Parklawn Drive ▼ Rockville, MD 20852 USA ◆ Telephone: (301)231-5510 Telex: 898-869 ATCCNORTH ▶ FAX: 301-770-2587

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Laboratory of Urological Oncology
Attn: Neil H. Bander, M.D.
Cornell Medical Center - Box 23
1300 York Avenue
New York, NY 10021

Deposited on Behalf of: Cornell University (c/o Dr. Neil H. Bander)

Identification Reference by Depositor:	ATCC Designation
Mouse hybridoma Prost E89	HB-12101

The deposits were accompanied by: ___ a scientific description _ a proposed taxonomic description indicated above.

The deposits were received X by this International Depository Authority and have been accepted.

AT YOUR REQUEST:

X We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested May 7, 1996. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Barbara M. Hailey

Date: May 8, 1996

Barbara M. Hailey, Administrator, Patent Depository

cc: Michael L. Goldman
Lauren S. Stich

American Type Culture Collection

12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)231-5520 Telex: 898-055 ATCCNORTH • FAX: 301-770-2587

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Cornell Medical Center
Attn: Dr. Neil H. Bander or Ms. Lauren Stich
Department of Urology
525 East 68 Street (Box 23, Rm. E-300)
New York, NY 10021

Deposited on Behalf of: Cornell Medical Center

Identification Reference by Depositor:	ATCC Designation
Mouse hybridoma prost J415	HB-12109

The deposit was accompanied by: __ a scientific description __ a proposed taxonomic description indicated above.

The deposit was received May 30, 1996 by this International Depository Authority and has been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested June 5, 1996. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Barbara M. Hailey

Barbara M. Hailey, Administrator, Patent Depository

Date: June 5, 1996

cc: Michael L. Goldman
H. Walter Haeussler

ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-365-2745

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Weill Medical College of Cornell University
Laboratory of Urological Oncology
Attn: Neil H. Bander
E-300
1300 York Avenue
New York, NY 10021

Deposited on Behalf of: Weill Medical College of Cornell University (Cornell Research Foundation)

Identification Reference by Depositor:	***Patent Deposit Designation***
Murine NSO myeloma cell line: 109BS J415 DIVH4 DIVK5	PTA-4174

The deposit was accompanied by: __ **a scientific description** _ **a proposed taxonomic description indicated above.**

The deposit was received March 21, 2002 **by this International Depository Authority and has been accepted.**

AT YOUR REQUEST: X **We will inform you of requests for the strain for 30 years.**

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested April 3, 2002. **On that date, the culture was viable.**

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Marie Harris

Marie Harris, Patent Specialist, ATCC Patent Depository

Date: April 16, 2002

cc: Louis Myers
(Ref: Docket or Case No.: 10448/163001 MPI01-140P3RCP1)

American Type Culture Collection

12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)231-5520 Telex: 908-768 ATCCHOVE • FAX: 301-816-4366

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

New York Hospital-Cornell Medical Center
Attn: Neil H. Bander, M.D.
Laboratory of Urological Oncology, Rm. E-300 (Box 23)
525 E. 68 Street
New York, NY 10021

Deposited on Behalf of: Dr. Neil H. Bander, Department of Urology, Cornell Medical Center

Identification Reference by Depositor:	ATCC Designation
Mouse hybridoma Prost J591	HB-12126
Mouse hybridoma Prost J533	HB-12127

The deposits were accompanied by: __ a scientific description __a proposed taxonomic description indicated above.

The deposits were received June 6, 1996 by this International Depository Authority and have been accepted.

AT YOUR REQUEST:

X We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested June 12, 1996. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Barbara M. Hailey

Barbara M. Hailey, Administrator, Patent Depository

Date: June 13, 1996

cc: ✓Michael L. Goldman
H. Walter Haeussler